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PERFUSION STUDIES OF THE HUMAN PLACENTA. III. PRODUCTION OF FREE AND CONJUGATED PORTER-SILBER CHROMOGENS*†

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ABSTRACT

Twenty human placentas obtained from normal pregnancies were perfused *in vitro* for six to twelve hours. The perfusion fluid (modified Tyrode's solution) was recycled at the rate of 175 ml. per minute and changed hourly. Aliquots of the hourly perfusates were assayed for free and conjugated Porter-Silber (P-S) chromogens. The hourly values for P-S chromogens were greater in the later hours of the perfusions. The cumulative values for P-S chromogens (expressed as cortisol equivalents) for individual perfused placentas varied from 0.305 mg. to 7.482 mg. free, and from 0.361 mg. to 6.025 mg. conjugated. The mean cumulative P-S chromogen per gram of placenta was 4.14 μg. free (S.E. = .865) and 4.79 μg. conjugated (S.E. = .632). These latter values were compared with the P-S chromogen content of 9 control unperfused placentas—0.37 μg. free (S.E. = .069) and 0.59 μg. conjugated (S.E. = .179) per gram of tissue. The differences between the amounts of P-S chromogens obtained from perfusates and that present initially in the control series was significant for both free ($P < .01$) and conjugated ($P < .001$) P-S chromogens. These results suggest production of free and conjugated P-S chromogens by the perfused placenta. The conjugated P-S chromogens were released by enzymic hydrolysis with mammalian β-glucuronidase. This hydrolysis could be inhibited by 1,4-saccharolactone. The conjugated P-S chromogens therefore appear to be in the form of glucosiduro-

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nates. The P-S chromogens in the perfusates included cortisol, cortisone, Reichstein's substance S, tetrahydrocortisone and tetrahydrocortisol, as indicated by countercurrent distribution, paper chromatography, blue tetrazolium reaction, ultraviolet absorption and sulfuric-acid chromogen curves. These 5 compounds appeared to account for 50–80 per cent of the total P-S chromogens measured.

THE changes in corticosteroid metabolism which take place during human pregnancy have been described in detail and the role of the placenta in these manifestations discussed at length (3). Most of the evidence relating to possible production of corticosteroids by the placenta has been indirect. The possibility of a direct approach to endocrine function of the placenta was suggested by our findings of metabolic activity and hormone responsiveness of the human placenta perfused *in vitro* (1, 4, 5). The present paper reports on the application of this technique to the study of possible corticosteroidogenesis by the placenta. The results suggest that the human placenta perfused *in vitro* may produce significant quantities of free and conjugated material with the characteristics of corticosteroids.

EXPERIMENTAL

The technique of placental perfusion developed for these studies has been reported in detail elsewhere (4). In brief, human placentas obtained from normal pregnancies are prepared for pulsatile retrograde perfusion with modified Tyrode's solution to which penicillin and streptomycin are added. The system is maintained at 37°C. The perfusing fluid is exposed to an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide and maintained at pH 7.4. One liter of perfusing fluid is recycled at a rate of about 175 ml. per minute and changed hourly during the perfusion. Perfusions are continued for varying periods up to twelve hours, after an initial "warm-up" perfusion interval which removes retained blood. The sterility of the perfusate is confirmed by frequent cultures.

Twenty placentas were perfused in this manner: 9 for ten to twelve hours, 7 for eight hours, and 4 for five to six hours. Duplicate 10-ml. aliquots of the hourly perfusates were assayed for Porter-Silber (P-S) chromogens by the method of Wu and Mason (6). The P-S chromogens are expressed as cortisol equivalents. The P-S chromogens extracted by dichloromethane before hydrolysis are referred to as "free"; those extracted after hydrolysis with mammalian β-glucuronidase are referred to as "conjugated." Enzymic hydrolysis was carried out at 37°C. following the addition of 3000 units of β-glucuronidase (Ketodase) to 10 ml. of solution buffered at pH 5.0. Inhibition experiments were performed by the addition of saccharolactone in varying amounts to the incubation mixture.

As controls, 9 additional placentas were individually homogenized immediately after the twenty-minute "warm-up" perfusion and before the regular perfusion was started. The tissue was thoroughly homogenized in perfusing fluid and duplicate 10-ml. aliquots of the aqueous extract were assayed for free and conjugated P-S chromogens as described. Previous experiments have shown no increased yield of P-S chromogens following initial homogenization with ethanol instead of perfusing fluid.

To obtain evidence for the nature of the Porter-Silber chromogens, extracts of larger volumes of perfusates were made with ethyl acetate before and after β-glucuronidase hydrolysis. The extract was partitioned between 70 per cent methanol and petroleum

ether, and the material from the methanol phase was characterized by various techniques.

Countercurrent distribution was performed using a 100-tube, all-glass automatic Craig-Post machine. Calculations were made according to Weisiger (7). The solvent systems used were those described by Carstensen (8) and Engel (9).

Paper chromatography was carried out using the technique and solvent systems described by Bush (10). Localization of steroids on paper chromatograms was accomplished by ultraviolet absorption and staining with blue tetrazolium. Elutions were performed by cutting the appropriate area into small strips, allowing the latter to stand in 25 ml. of methanol overnight and then extracting twice again with methanol the following morning.

Sulfuric acid chromogen curves were obtained as described by Zaffaroni (11).

RESULTS

Amount of P-S chromogens in perfusates and control placentas

The amount of free P-S chromogens in each hourly perfusate of the 20 perfused placentas is indicated in Table 1; the values for conjugated P-S chromogens are recorded in Table 2. The mean values for all perfusions of the amount of P-S chromogens present at each hour in the placental perfusate are recorded in the bottom row of Tables 2 and 3. The amounts of P-S chromogens in the perfusate were increased in the later hours of the perfusions; this was more marked with the free chromogens. The right-hand columns of Tables 1 and 2 list the cumulative or total P-S chromogens, free and conjugated respectively, obtained from individual placentas. The cumulative values varied from .305 mg. to 7.482 mg. free, and .361 mg. to 6.025 mg. conjugated. The mean value for cumulative P-S chromogens per perfused placenta was 2.559 mg. free and 2.959 mg. conjugated.

The average total yield of P-S chromogens from the placental perfusates was 4.14 μg. free (S.E. = .865) and 4.79 μg. conjugated (S.E. = .632) per gram of placenta. In contrast were the amounts of P-S chromogens present in 9 placentas which were not perfused. These values are shown in Table 3. The average concentration of P-S chromogens in the unperfused placenta was 0.37 μg. free (S.E. = .069) and 0.59 μg. (S.E. = .179) conjugated per gram of placenta. The differences between the amounts of P-S chromogens obtained from perfusates and that present initially in the control series of unperfused glands was significant for both free ($P < .01$) and conjugated ($P < .001$) values.

Control studies showed no P-S chromogens present in the perfusing fluid itself following control runs through the perfusion apparatus at 37°C.

Nature of P-S chromogens

All the P-S chromogens obtained from perfusates exhibited the peak absorption at 410 mμ characteristic of corticosteroids with a 17,21-dihy-

TABLE 1. FREE PORTER-SILBER CHROMOGENS IN EACH HOURLY PERFUSATE (MG. OF CORTISOL EQUIVALENTS)

Placenta No.	Wt. of placenta (Gm.)	Hours perfused	Perfusion hour												Total
			1	2	3	4	5	6	7	8	9	10	11	12	
1	538	12		.272	.058	.318	.490	.580	1.110	.796	.485	.674	.876	1.030	6.689
2	767	12			.316		.555	1.250	.631	.773	1.555		.462	1.940	7.482
3	505	12	.058	.081	.029	.153	.183	.167	.407	.511	1.020	.879	1.030	.932	5.450
4	700	12		.531	.278	.101	.061	.235	.272	.273	.215	.247		.259	2.472
5	490	12			.274	.034	.078	.040		.149	.324	.363	.268	.432	1.962
6	704	11	.308	.140	.372		.320		.189	.650	.475	.980	1.950		5.384
7	594	11	.204	.077	.188	.144	.102	.162	.202	.150	.090	.154	.132		1.605
8	738	11		.294		.177	.081	.164		.374	.041	229	.275		1.635
9	745	10	.247	.067	.098	.088	.122	.050	.031	.065	.112	.155			1.030
10	546	8	.007	.042	.046	.029	.088	.026	.050	.033					.321
11	595	8	.004	.086	.352	.322	.407		.619	1.033					2.823
12	477	8		.491		.407	.066	.106	.094	.151					1.315
13	830	8	.319	.232	.247	.230	.211	.103	.079	.169					1.590
14	580	8	.279	.280	.215	.199	.205	.128	.186	.192					1.686
15	620	8	.025	.034	.040	.028	.048	.051	.031	.048					.305
16	600	8	.162	.266	.165	.130	.182	.158	.197	.259					1.519
17	730	6	.119	.338	.558	.345	.124	.034							1.518
18	600	6	.212	.262	.172	.186	.197	.245							1.274
19	471	6	.071	.236	.325			.329							.961*
20	434	5		.066	.194	.093	.233								.586*
Mean	613		.155	.210	.218	.175	.198	.225	.292	.352	.480	.460	.713	.919	2.559

Mean total Porter-Silber chromogen in μg. per Gm. of placenta = 4.14 (S.E. = .865)

* Not included in total average because only 4 hourly values available.

TABLE 2. CONJUGATED PORTER-SILBER CHROMOGENS IN EACH HOURLY PERFUSATE (MG. OF CORTISOL EQUIVALENTS)

Placenta No.	Wt. of placenta (Gm.)	Hours perfused	Perfusion hour												Total
			1	2	3	4	5	6	7	8	9	10	11	12	
1	538	12		.072	.116	.176	212	.249	.212	.252	.301	.296	.338	.336	2.560
2	767	12			.016		.689	.241	.345	.469	.366		1.454	.600	4.180
3	505	12			.284		.480		.267		.500	.544	.494	.958	3.527
4	700	12	.675	.549	.422	.232	.194	.255	.343	.222	.221	.481		.225	3.819
5	490	12			.481	.048	.356	.107		.246	.706	.198	.741	.346	3.229
6	704	11	.716	.412	.382	.192	.349	.145	.464	.491	.745	.945	.782		5.623
7	594	11	.261	.424	.462	.394	.464	.467	.406	.711	.868	.764	.804		6.025
8	738	11		.497		.175	.494	.274		.429	.225	.494	.446		3.034
9	745	10	.376	.176	.145	.202	.123	.211	.257	.338	.167	.245			2.240
10	546	8	.023	.061	.022	.134	.078	.069	.058	.277					.722
11	595	8				.165	.181	.315	.330	.329					1.320
12	477	8		.534		.432	.261	.384	.198	.228					2.037
13	830	8	.245	.260	.260	.280	.208	.248	.236	.341					2.078
14	580	8	.312	.513	.417	.548	.577	.617	.719	.876					4.579
15	620	8	.130	.043	.118	.090	.065	.247	.130	.110					.933
16	600	8	.359	.426	.366	.552	.353	.267	.546	.428					3.297
17	730	6			.053	.429	.152	.099							.733
18	600	6	.354	.524	.561	.509	.704	.675							3.327
19	471	6	.061	.072	.076			.152							.361*
20	434	5		.131	.648	.183	.541								1.503*
Mean	613		.284	.313	.284	.279	.341	.279	.322	.383	.467	.496	.723	.493	2.959

Mean total Porter-Silber chromogen in μg. per Gm. of placenta = 4.79 (S.E. = .632)

* Not included in total average because only 4 hourly values available.

TABLE 3. PORTER-SILBER CHROMOGENS EXTRACTED FROM CONTROL PLACENTAS (EXPRESSED AS CORTISOL EQUIVALENTS)

Placenta No.	Wt. of placenta (Gm.)	P-S chromogens (μg. per Gm. of placenta)	
		Free	Conjugated
1	765	.525	.284
2	566	.111	.544
3	866	.600	1.587
4	743	.387	.506
5	591	.047	.194
6	637	.646	.172
7	575	.177	.356
8	501	.347	.245
9	761	.480	1.431
Mean	667	.369	.591
S. E. of mean		.069	.179

droxy-20-keto configuration. The nature of the P-S chromogens in the perfusates was studied by analysis of the extracts obtained from selected large aliquots of perfusate. Evidence was obtained for the presence of the following 5 steroids (Table 4):

Δ^4-Pregnene-11β,17α,21-triol-3,20-dione (cortisol). P-S positive material had partition coefficients in two countercurrent distribution systems and mobilities in two successive paper chromatography systems identical with those of standard cortisol. The P-S material was ultraviolet-absorbing and blue-tetrazolium positive. A sulfuric-acid chromogen curve was in agreement with that of standard cortisol.

Δ^4-Pregnene-17β,21-diol-3,11,20-trione (cortisone). The unknown material was shown to be ultraviolet-absorbing and blue-tetrazolium positive and to have mobilities identical with those of standard cortisone in three successive paper chromatography systems. The cortisone-like material was

TABLE 4. EVIDENCE FOR CORTICOSTEROIDS IN PLACENTAL PERFUSATE

Compound	B.T.*	U.V.**	Rf in paper chromatography†			Partition coefficient (K) in countercurrent distribution†		
			I	II	II	IV	V	VI
Cortisol	+	+	.34 [.34]‡	.34 [.34]		5.7 [5.9]		.33 [.32]
Cortisone	+	+	.53 [.52]	.50 [.48]	.10 [.10]	2.7 [2.8]		.82 [.79]
Substance S	+	+	.81 [.83]		.40 [.39]			14 [13.0]
Tetrahydrocortisone	+	−	.28 [.28]	.30 [.31]			1.00 [0.98]	
Tetrahydrocortisol	+	−	.17 [.16]	.20 [.20]			1.8 [1.8]	

* Blue tetrazolium.
** Ultraviolet-absorbing.

† I = $\frac{\text{Benzene}}{\text{50\% } CH_3OH : \text{50\% } H_2O}$ Bush B-5 (10).

II = $\frac{\text{90\% Toluene : 10\% Ethyl acetate}}{\text{50\% } CH_3OH : \text{50\% } H_2O}$ Bush C (10).

III = $\frac{\text{67\% Toluene : 33\% Ligroin}}{\text{70\% } CH_3OH : \text{30\% } H_2O}$ modified Bush B-2 (10).

†IV = $\frac{\text{70\% } CH_3OH : \text{30\% } H_2O}{\text{30\% } CHCl_3 : \text{70\% } CCl_4}$ (9).

V = $\frac{\text{70\% } CH_3OH : \text{30\% } H_2O}{\text{70\% } CHCl_3 : \text{30\% } CCl_4}$ (9)

VI = $\frac{\text{Benzene}}{\text{.05\% NaCl}}$ (8).

‡ Values in brackets are those for the authentic reference standard.

P-S positive and had partition coefficients identical with those of standard cortisone in two countercurrent distribution systems.

Δ^4-Pregnene-17α,21-diol-3,20-dione (substance S). P-S positive material had a partition coefficient identical with that of standard substance S in one countercurrent distribution system. Blue-tetrazolium positive, ultraviolet-absorbing material had mobilities identical with those of standard substance S in two sequential paper chromatography systems.

5β-Pregnane-3α,17α,21-triol-11,20-dione (tetrahydrocortisone, THE). P-S material had a partition coefficient identical with that of standard THE in one countercurrent distribution. Blue-tetrazolium positive, non-ultraviolet-absorbing material had mobilities identical with those of standard material in two sequential paper chromatography systems.

5β-Pregnane-3α,11β,17α,21-tetrol-20-one (tetrahydrocortisol, THF). P-S material had a partition coefficient identical with that of standard THF in one countercurrent distribution system. Blue-tetrazolium positive, non-ultraviolet-absorbing material had mobilities identical with those of standard THF in two sequential paper chromatography systems.

TABLE 5. NATURE OF PORTER-SILBER CHROMOGENS IN PLACENTAL PERFUSATES

Perfusion No.	Hour No.	Aliquot (ml.)	Tentatively identified (μg.)					Unidentified (μg.)
			Cortisol	Cortisone	Substance S	THF	THE	
6	11	200	120	100	60	75	40	115
11	8	250	85	55	40	30	30	90
18	6	200	35	15		50	20	100

That other compounds than these 5 were present in the perfusate is indicated by the finding that the amount of P-S material accounted for by these compounds ranged only from 50 to 80 per cent of the total P-S chromogens present in the perfusate (Table 5). Other polar P-S positive material was present in the chromatograms, as well as other blue-tetrazolium positive material.

The effect of 1,4-saccharolactone, an inhibitor of β-glucuronidase activity (12), on enzymic hydrolysis of the perfusate is shown in Table 6. Saccharolactone was added to incubation mixtures of perfusate from which free P-S chromogens had been extracted. It may be seen that no significant release of P-S chromogens took place following incubation of this perfusate at 4°C. P-S chromogens were released after incubation at 37°C. without the addition of β-glucuronidase. This release, which was progressively inhibited by increasing amounts of saccharolactone, was probably due to the presence of β-glucuronidase activity in the placental perfusate (unpublished observations). Addition of β-glucuronidase to the incubation mixture aug-

mented the release of P-S chromogens and this hydrolysis also could be progressively inhibited by increasing amounts of saccharolactone.

DISCUSSION

In our initial studies of possible corticosteroidogenesis by the human placenta, perfusion was carried out for only two to four hours. Free Porter-Silber chromogens were measured in the perfusate and in small segments of the placental tissue before and after the perfusion. The amount of P-S chromogens in the perfusate rarely was significantly more than could be accounted for by release of preformed P-S chromogens from the placental

TABLE 6. PORTER-SILBER CHROMOGENS (EXPRESSED IN CORRECTED OPTICAL DENSITY) FOLLOWING INCUBATION UNDER VARYING CONDITIONS OF 10-ML. ALIQUOTS OF PERFUSATE FROM WHICH FREE P-S CHROMOGENS HAD PREVIOUSLY BEEN EXTRACTED

	At 4°C.	At 37°C. without added β-glucuronidase				At 37°C. with added β-glucuronidase			
Saccharolactone (mg.)	0	0	20	40	80	0	20	40	80
Corrected optical density	.012	.135	.145	.051	.028	.452	.309	.175	.058

Incubator: 18 hours, acetate buffer, pH 5.0, 3000 units Ketodase.

tissue. To avoid, if possible, such a result, the present study was undertaken with longer perfusion periods and with measurement of both free and conjugated P-S chromogens. For a more reliable control than small sections of perfused placentas, a series of intact placentas were assayed for P-S chromogen content. The control values obtained in this way agreed with the earlier control values. Although no recovery experiments were performed, there are good reasons for believing that the control values were not a gross underestimate. Other investigators have reported quantitative studies of placental corticosteroid content. Berliner *et al.* (13) isolated 10 μg. of corticosteroids per 100 Gm. of tissue; of this, cortisone constituted 9 μg. Sybulski and Venning (14) found between 8.9 and 33.0 μg. of cortisone per 100 Gm. of placental tissue; cortisol was found in much smaller amounts. The control values in the present study (37 μg. of free, and 59 μg. of conjugated P-S chromogens per 100 Gm. of placental tissue) therefore seem to provide a safe basis from which to estimate a major production of this material during perfusion. Such a method of estimating production presupposes that there is no significant amount of preformed P-S chromogen bound to placental tissue in a form which is disrupted only during perfusion and not during control tissue extractions.

The amounts of P-S chromogens in the perfusates of placentas perfused *in vitro* for six to twelve hours were significantly greater than the values of

P-S chromogens in control placentas. With the foregoing reservation, this suggests production of these P-S chromogens by the placenta during perfusion. Both free and conjugated P-S chromogens were significantly increased in the perfusate compared with the control starting values. There was no uniform response, however, by the various placentas; a wide range of P-S chromogen output was noted. This seemed to be due, at least in part, to the varying lengths of perfusion time. The variability in yield of P-S chromogens may also be attributable to other factors, as indicated by the considerable variation between placentas at given hours. Even more striking was the marked increase in apparent P-S chromogen production during the later hours of the perfusion. It is possible that some adaptation process or anoxia may be involved. It is noteworthy that the action of estradiol and human chorionic gonadotropin on citrate utilization by the perfused placenta becomes apparent only after a five-hour lag (4), whereas conversion of estradiol to estriol following the addition of human chorionic gonadotropin can be demonstrated during the initial hours of placental perfusion (5).

Studies with β-glucuronidase hydrolysis and saccharolactone inhibition suggest that the conjugated P-S chromogens are in the form of glucosiduronates. Since the conjugated P-S chromogens also show a significant increase during perfusion it appears that the placenta is capable of conjugating this material as glucosiduronates. Although this has not been shown for other substrates such as bilirubin (15), it is possible that the same enzyme system does not serve different substrates.

Although the P-S chromogens were not isolated in crystalline form, evidence was obtained indicating the presence of cortisone, cortisol, substance S, tetrahydrocortisone and tetrahydrocortisol. These 5 compounds account only for 50–80 per cent of the P-S material measured; the nature of the other 20–50 per cent is not yet known. It is possible that the other polar material noted after ethyl acetate extraction may be 6-hydroxylated corticosteroids (16). It should be emphasized that in the absence of complete identification of all the P-S chromogens, it cannot be stated whether the products of perfusion are corticosteroids or only P-S chromogens similar to corticosteroids.

Conflicting results have been reported by workers studying the *in vitro* capacity of the human placenta to produce corticosteroids. Sybulski (17) and Sybulski and Venning (14) incubated minces of placental tissue and found no production of cortisone, cortisol or their reduced metabolites. Solomon (18) incubated placental homogenates and also found no evidence for corticosteroid production. Little and Rossi (19) incubated placental tissue in a mixture of Krebs-Ringer solution and maternal serum and measured the free P-S chromogens in the medium as well as in the placental tissue before and after incubation. An increase in P-S chromogens was

demonstrated in the medium without a significant loss of such unconjugated material from the homogenates. Romanoff (20) perfused isolated placentas with whole beef blood for periods up to four hours. The results suggested that the perfused placenta was able to produce Δ^4-3-ketones, but the alternative possibility of release of preformed steroids could not be ruled out. Endröczi *et al.* (21) found that incubation of placental tissue with progesterone yielded large amounts of cortisone, cortisol and corticosterone. These compounds were identified by their Rf values before and after acetylation, by fluorescence with alkali, and by the tetrazolium reaction.

Although the demonstration of corticosteroid biogenesis in the placenta would be of interest, it is clear that during pregnancy in adrenal-deficient women there is generally insufficient adrenocortical hormone activity to protect the mother against the demands of pregnancy and its associated stresses (see review (3)).

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17α-HYDROXYPREGNENOLONE AS A PRECURSOR OF URINARY STEROIDS IN A PATIENT WITH A VIRILIZING ADENOMA OF THE ADRENAL

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ABSTRACT

By isotopic techniques, the daily secretory rate of 17α-hydroxypregnenolone in a patient with a virilizing adrenal adenoma has been estimated to be 63 mg. per day. This steroid, and not 17α-hydroxyprogesterone, was found to be the principal precursor of the urinary metabolite, pregnane-3α,17α,20α-triol. The data also demonstrated that only a small fraction of the 110 mg. of dehydroisoandrosterone found in a daily urine sample from this patient was derived from the peripheral conversion of 17α-hydroxypregnenolone.

THIS paper describes the attempts made to trace the adrenal precursors of certain urinary steroids excreted by a patient with a virilizing adrenal adenoma.

Dehydroisoandrosterone sulfate (DHS)[1] is a constituent of normal male and female urine and is present in greatly increased amounts in the urine of patients with virilization due to adrenal tumors. Until recently, little was known about the steroidal precursor(s) of this urinary metabolite. Dehydroisoandrosterone (D) has been isolated from human adrenal venous blood by Bush and Mahesh (1), by Lombardo *et al.* (2), and most recently by Hirschmann *et al.* (3); therefore, it can be assumed that it is the precursor of at least a part, if not most, of the urinary DHS. In several instances, however, D was found to be present in adrenal venous blood in such small concentrations that it was doubtful that the amounts were

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[1] The abbreviations and trivial names used in this paper are as follows:
D = dehydroisoandrosterone, 3β-hydroxy-Δ⁵-androsten-17-one.
DHS = dehydroisoandrosterone sulfate.
17α-Hydroxypregnenolone = 3β,17α-hydroxy-Δ⁵-pregnen-20-one.
A = androsterone, 3α-hydroxyandrostan-17-one.
E = etiocholanolone, 3α-hydroxyetiocholan-17-one.
Δ⁵-Pregnenetriol = Δ⁵-pregnene-3β,17α,20α-triol.
Pregnanetriol = pregnane-3α,17α,20α-triol.
17α-Hydroxyprogesterone = 17α-hydroxy-Δ⁴-pregnene-3,20-dione.

sufficient to account for most of the DHS observed in the urine (1, 2). Some years ago, it was suggested (4) that the urinary DHS might originate by peripheral conversion from 17α-hydroxypregnenolone, itself secreted by the adrenal glands. In support of this hypothesis, Carstensen *et al.* (5) demonstrated that 17α-hydroxypregnenolone could be detected in dog adrenal vein blood, whereas D was either absent, or present only in insignificant amounts. Since the claim had been made earlier (6) that intravenous infusion of 17α-hydroxypregnenolone results in an increase in the plasma level of D in the adrenalectomized dog, it was concluded that, at least in this animal, D was not an adrenal secretory product but was derived from 17α-hydroxypregnenolone by peripheral cleavage of the side-chain.

In the human, *in vivo* conversion of 17α-hydroxypregnenolone to urinary DHS was demonstrated by Solomon *et al.* (7). These investigators isolated labeled D from the urine of a patient with metastatic adrenal disease to whom tritiated 17α-hydroxypregnenolone had been administered. This experiment revealed that 2.8 per cent of the C_{21} precursor had been converted to the C_{19} metabolite. Since the amount of the C_{21} precursor secreted by the tumor was unknown, it was not possible to estimate the extent to which the peripheral metabolism of 17α-hydroxypregnenolone accounted for the observed urinary level of D.

In order to estimate the secretory rate of 17α-hydroxypregnenolone and its contribution, by way of peripheral conversion to the urinary C_{19} steroids, the following experiment was carried out: Tritium-labeled 17α-hydroxypregnenolone was administered to a female with a virilizing tumor of the adrenal. Tritiated dehydroisoandrosterone (D), androsterone (A), etiocholanolone (E), Δ^5-pregnene-3β,17α,20α-triol and pregnane-3α,17α, 20α-triol were isolated from the urine. The secretory rate of 17α-hydroxypregnenolone was calculated from the observed specific activity of the isolated Δ^5-pregnenetriol. The data obtained by means of these isotopic techniques indicated that the amount of 17α-hydroxypregnenolone secreted each day by this patient was 63 mg. Moreover, this steroid, and not 17α-hydroxyprogesterone, was the principal precursor of the urinary metabolite, pregnane-3α,17α,20α-triol. The results also demonstrated that, although more than 100 mg. of dehydroisoandrosterone was excreted in the urine of this patient, only a small fraction of it was derived through the peripheral conversion of 17α-hydroxypregnenolone.

MATERIAL AND METHODS

General methods

All radioactive samples were counted in the Packard Tri-Carb liquid scintillation spectrometer Model 314-DC, with the counting chamber set at −2°C. The samples to be counted were transferred to 5-dram vials (Wheaton Glass Co.) and dissolved in 5 ml.

of toluene containing 0.3 per cent of 2,5-diphenyl-oxazole and 0.01 per cent of 1,4-bis-2(5-phenyl-oxazolyl)-benzene (Pilot Chemicals). Tritium was counted at high voltage tap 8 (1200 volts) with the pulse height discriminator set at 60 and 100 volts. At this reading the efficiency of tritium counting was approximately 20 per cent. A sealed standard of tritiated cholesterol was used as point of reference. All final counts were performed for a sufficient length of time to give a standard error of not more than 5 per cent. Impure samples were checked for quenching, and correction for this was made when present.

All melting points were obtained on a Köfler block and were corrected. Infrared spectra were determined using a model 21C Perkin-Elmer double-beam spectrophotometer.

The patient

The patient was a 26-year-old virilized female who excreted in the urine 132 mg. of 17-ketosteroids, 4.4 mg. of pregnanetriol, 7.7 mg. of 17-hydroxycorticosteroids and less than 6 international units of follicle-stimulating hormone (FSH) per twenty-four hours.

TABLE 1. RADIOHOMOGENEITY OF INJECTED 17α-HYDROXYPREGNENOLONE ACETATE

Crystallization No.	Solvent	Crystalline product		Mother liquor, specific activity (cpm/mg.)
		Weight (mg.)	Specific activity (cpm/mg.)	
1	Acetone	73.6	2.1×10^4	1.7×10^4
2	MeOH:Acetone	56.6	2.0×10^4	2.0×10^4
3	MeOH:Acetone	42.8	2.0×10^4	1.9×10^4
4	MeOH:Acetone	37.5	1.9×10^4	1.9×10^4

In the resting state, the level of plasma free 17-hydroxycorticoids was 16 μg. per 100 ml., and rose to 46 μg. per 100 ml. after a four-hour intravenous infusion of 25 mg. of adrenocorticotropin (ACTH).

At operation a right-sided adrenal adenoma weighing 33 grams was removed. The contralateral adrenal was not exposed. Microscopic examination of the tumor revealed massive hyperplasia of the zona reticularis, with variation in cell size but without evidence of malignancy. The zona glomerulosa and fasciculata appeared quite normal. Eight days after operation the urinary 17-ketosteroids averaged 10 mg. per twenty-four hours.

Preoperatively, tritiated 17α-hydroxypregnenolone (11 million counts per minute, specific activity 2.5 mc. per mg.) was injected intravenously, and thereafter urine was collected for four days. The injected material (17α-hydroxypregnenolone-7α-H^3) was purchased from the New England Nuclear Corporation. In order to check its radiochemical homogeneity, 100 mg. of unlabeled 17α-hydroxypregnenolone was added to a portion of the radioactive material. The mixture was acetylated and recrystallized repeatedly from methanol-acetone. The specific activities of the crystals and the mother liquor obtained after each crystallization are shown in Table 1.

Isolation of urinary steroids

Each of the four-day urine collections was adjusted to pH 5.0 with 50 per cent sulfuric acid, and acetate buffer was added. Mammalian β-glucuronidase (300,000 units) was added and each sample was incubated for four days at 37°C. The liberated steroids

TABLE 2. RECOVERY OF RADIOACTIVITY FROM URINE AFTER HYDROLYSIS OF STEROIDS

Day	Enzyme hydrolysis		Acid hydrolysis		Total, enzyme+acid	
	% of injected dose	% total excreted	% of injected dose	% total excreted	% of injected dose	% total excreted
1	15.0	61	16.0	83	31	70
2	6.0	25	2.6	13	9	20
3	2.6	11	0.7	3	3	8
4	0.8	3	0.3	1	1	2
Total	24.4	100	19.6	100	44	100

were exhaustively extracted with ether. The ether extract was washed with 5 per cent sodium hydroxide and water and then evaporated to dryness in preparation for radioactive assay. Steroids in the spent urine after extraction were hydrolyzed further by the solvolysis procedure of Burstein and Lieberman (8). The urine for each day was treated separately. The recovery of radioactivity in the extracts after enzyme and acid hydrolysis is shown in Table 2. The enzyme-hydrolysis extracts for days 1 to 4 were pooled, as were the acid-hydrolysis extracts. Half of each pool was chromatographed on a 20-gram column of silica gel, using 100-ml. portions of eluant. The distribution of counts after chromatography is shown in Tables 3 and 4.

Dehydroisoandrosterone (D)

In order to determine the total amount of D excreted during the four-day period, one-thirtieth of fractions 4 and 5 (Table 4) was analyzed quantitatively by chromatography on alumina according to the gradient elution method of Lakshmanan and Lieberman (9). A single peak was obtained which was identified as dehydroisoandrosterone by paper chromatography, sulfuric-acid chromogen and infrared analysis. Using the method of Bitman *et al.* (10), isoandrosterone was sought for in this fraction but could not be detected. In this way, the daily excretion of D during the collection period was estimated to be 110 mg. From the remainder of fraction 4 (Table 4), dehydroisoandrosterone was obtained by crystallization, and identified by its melting point and infrared spectrum. Its specific activity was 236 cpm per mg. After recrystallization from methanol-ether, the specific activity was 233 cpm. per mg.

TABLE 3. SILICA-GEL CHROMATOGRAM AFTER ENZYME HYDROLYSIS OF STEROIDS (POOLED URINE)

Fraction	Eluant (% EtOH in benzene)	Radioactivity (total cpm)	Weight (mg.)
1	—	—	5
2	0.5	—	6
3	1	—	1
4	2	113,300	84
5	3	113,400	37
6	4	119,000	19
7	5	196,500	12
8	10	498,000	25
9	20	158,000	27
		Total 1,198,200	

Androsterone (A) *and etiocholanolone* (E)

Fractions 4, 5 and 6 (Table 3) (weight 140 mg., radioactivity 345,000 cpm) were combined and chromatographed on alumina using gradient elution (9). Two major peaks were obtained. The androsterone fraction weighed 14.4 mg. and had a specific activity of 870 cpm per mg. This fraction was further purified by chromatography on alumina and by crystallization (twice) from acetone. The product melted at 177–180° (authentic m.p. 185°) and possessed an infrared spectrum identical with that of androsterone. Its final specific activity was 900 cpm per mg. The daily excretion was 7 mg.

The second peak contained 43.8 mg. (by weight) of etiocholanolone with a specific activity of 2890 cpm per mg. After further purification on alumina and two crystallizations from Skellysolve C:benzene, a product melting at 141–143° (authentic m.p. 143–144°) and having an infrared spectrum identical with that of etiocholanolone was obtained. Its final specific activity was 3700 cpm per mg. The daily excretion was 20 mg.

Pregnane-3α,17α,20α-triol and Δ⁵-pregnene-3β,17α,20α-triol

Fractions 7, 8 and 9 (Table 3) were rechromatographed on silica gel using methanol: water as stationary phase, and ethyl acetate:isooctane as mobile phase (11). One crystalline fraction weighing 38 mg. and containing 870,000 cpm was obtained. As evidenced by the melting point (m.p. 229–232°), this material appeared to be a mixture, and further purification was effected by digitonin precipitation. After cleavage of the digitonide with pyridine the *beta* fraction weighed 4.8 mg. and contained 230,000 cpm. Recrystallization from ethyl acetate:acetone gave 2.6 mg. of a product melting at 217–221° (authentic Δ⁵-pregnene-3β,17α,20α-triol melts at 219–222°C).[2] This substance was acetylated and the product chromatographed on alumina. The material eluted with benzene was recrystallized from methyl alcohol:acetone and yielded 2 mg. of Δ⁵-pregnene-3β,17α,20α-triol diacetate, m.p. 203–205° (m.p. of authentic sample, 203–205°). Its infrared spectrum was identical with that of the authentic diacetate. The specific activity, calculated as the free compound, was 39,000 cpm per mg. It is noteworthy that this sample of Δ⁵-pregnenetriol was isolated from the enzyme-hydrolyzed fraction, albeit in smaller amount than was recovered from the acid-hydrolyzed fraction.

The *alpha* fraction from the digitonin precipitation weighed 21 mg. and contained 450,000 cpm. After chromatography on silica gel, 10 mg. of pregnanetriol melting at 243–246° (authentic pregnane-3α,17α,20α-triol melts at 251–252°) was obtained. After acetylation, chromatography of the diacetate on alumina and saponification, 6 mg. of pregnane-3α,17α,20α-triol (m.p. 246–249°) was recovered. On admixture with the authentic triol, no depression in the melting point was observed. After recrystallization from methanol, the specific activity was 41,800 cpm per mg. The infrared spectrum (in KBr) was identical with that of the known sample.

Fractions 7, 8 and 9 (Table 4) were acetylated and chromatographed on a 10-gram alumina column. A single fraction, weighing 25 mg., was eluted with 10 per cent ethyl acetate in benzene. After recrystallization from methanol:ligroin, 9.4 mg. of Δ⁵-pregnene-3β,17α,20α-triol-diacetate was obtained. The melting point was 202–204°, and the specific activity 43,200 cpm per mg., calculated as the free steroid. Identity was confirmed by infrared analysis.

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[2] The authentic sample of Δ⁵-pregnenetriol was obtained through the courtesy of Dr. H. Hirschmann.

TABLE 4. SILICA-GEL CHROMATOGRAM AFTER SOLVOLYSIS OF STEROIDS (POOLED URINE)

Fraction	Eluant (% EtOH in benzene)	Radioactivity (total cpm)	Weight (mg.)
1	—	—	—
2	0.5	—	—
3	1	—	—
4	2	91,300	340
5	3	73,100	74
6	4	101,200	26
7	5	517,100	28
8	10	371,100	40
9	20	61,000	34
10	100	22,800	—
		Total 1,237,600	

RESULTS AND DISCUSSION

Since it is reasonable to assume that Δ^5-pregnenetriol is derived solely from 17α-hydroxypregnenolone, the specific activity of this metabolite was used for the calculation of the secretory rate of 17α-hydroxypregnenolone by the formula:

$$\text{Secretory rate in mg. per day} = \frac{\text{counts injected}}{\text{specific activity of urinary metabolite} \times \text{days of urine collection}}.$$

The contribution of endogenously secreted 17α-hydroxypregnenolone to the other urinary steroids was estimated with the use of an approach discussed in an earlier paper (12). Provided certain conditions are met, the assumption was made that any urinary metabolite having the same specific activity as the isolated Δ^5-pregnenetriol can have been derived only from 17α-hydroxypregnenolone. Precursors other than this C_{21} steroid can not have contributed significant amounts to the excretion of this urinary metabolite. On the other hand, a metabolite containing tritium but having a specific activity lower than that of the isolated Δ^5-pregnenetriol is derived not only from 17α-hydroxypregnenolone but also from other precursors. As previously emphasized (12), although the differences in the specific activity are directly proportional to the contribution of the unknown precursors to the urinary metabolite, these differences furnish no information about the actual amount of the unknown precursor produced endogenously. To estimate this quantity, the identity of the unknown and the extent of its conversion to the urinary metabolite must be known.

Figure 1 illustrates the pathways by which some of the steroids synthesized by the patient under consideration were catabolized. In this figure the

products actually secreted by the adrenal are distinguished from compounds formed from the secretory products by reactions occurring outside the adrenal glands. Of the five secretory products shown at the top, three, (Δ^4-androstenedione, testosterone, and 17α-hydroxyprogesterone) are enclosed in brackets to indicate that they were produced by this patient in much smaller amounts than were dehydroisoandrosterone and 17α-hydroxypregnenolone (see subsequent data). Following their release from the glands, the secretory products may be interconverted as indicated and then catabolized to compounds which are excreted in the urine conjugated as sulfates or glucuronidates. The final specific activities of the urinary metab-

TABLE 5. DISTRIBUTION OF RADIOACTIVITY IN THE ISOLATED URINARY STEROIDS

Steroid	Daily Excretion (mg.)	Recovery of Injected Dose (%)	Specific activity (cpm/mg.)
Dehydroisoandrosterone (D)	110	0.95	233
Androsterone (A)	7	0.24	900
Etiocholanolone (E)	20	2.8	3,700
Pregnanetriol	4	6.4	41,800
Δ^5-Pregnenetriol	13	22.0	43,000

olites isolated after the administration of tritiated 17α-hydroxypregnenolone, as well as the percentage yield of the metabolites from this precursor, are recorded in Table 5.

Of the injected radioactivity, 22 per cent was recovered in the urine as Δ^5-pregnenetriol, the daily excretion of which was 13 mg. From the cumulative specific activity of this metabolite, a secretory rate for 17α-hydroxypregnenolone of 63 mg. per day was estimated.

Δ^5-Pregnenetriol is excreted in small amounts in normal urine (13–16), and it must therefore be assumed that 17α-hydroxypregnenolone is normally secreted by the adrenal. The normal urinary levels of the triol have been estimated to be 0.30 ± 0.05 mg. per day by Nowaczynsky *et al.* (14), and 0.06–0.20 mg. per day by Cox and Shearman (17). Assuming the same percentage conversion (22 per cent), the secretory rate of 17α-hydroxypregnenolone in the normal subject can be estimated from these urinary levels to be in the order of 0.3–1.4 mg. per day. This is a minimal figure, since it is likely that the percentage conversion in a healthy person would be smaller than it is in a patient secreting 63 mg. per day.

Six per cent of the radioactivity administered was found in the urinary pregnanetriol which was excreted in amounts of 4 mg. per day. Since the specific activity of this triol was insignificantly different from that of the isolated Δ^5-pregnenetriol, it may be concluded that both triols were derived

solely from a common precursor. As discussed previously, this precursor must be 17α-hydroxypregnenolone. Thus, it is evident that 17α-hydroxyprogesterone contributed little or nothing to the urinary pregnanetriol excreted by this patient. In view of the fact that Fotherby and Love (16) observed that the Δ^4-3-ketone is a more efficient precursor of pregnanetriol than is 17α-hydroxypregnenolone, it is further apparent that had even small amounts of 17α-hydroxyprogesterone been synthesized, its contribution to urinary triol would have been easily detected. Thus, the secretory rate of 17α-hydroxyprogesterone in this patient must have been nil, or at least insignificant, in comparison to that of 17α-hydroxypregnenolone.

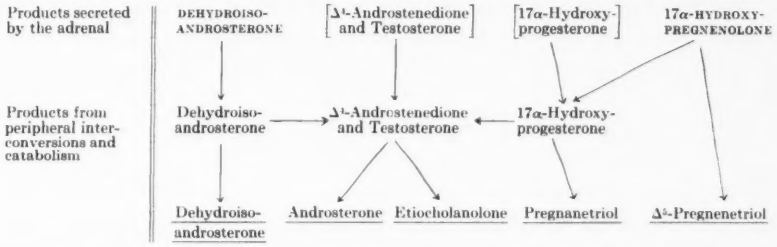

FIG. 1. Peripheral interconversions and catabolic fate of steroids secreted by an adrenal adenoma. Underlined compounds denote isolated urinary metabolites.

Pregnanetriol is also a normal constituent of urine, estimated to be excreted in amounts of 0.5 to 1.5 mg. per day (15). It is evident from our findings that, unless the relative contributions of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are known, the actual precursor of this urinary metabolite in the normal subject can not be assumed. These considerations are especially pertinent in diseases such as congenital adrenal hyperplasia, in which urinary pregnanetriol levels are greatly elevated (18, 19). Since the hormonal precursor of pregnanetriol is uncertain, speculation about the existence of possible defects in processes involved in the biogenesis of the adrenal secretory products is unwarranted unless the contributions of each of its precursors have been established in every instance.

Only about 1 per cent of the injected radioactivity was found in the urinary D, even though 110 mg. of this 17-ketosteroid was excreted daily by this patient. The specific activity of the isolated D was only 233 cpm per mg., from which it can be calculated that only about 0.5 per cent (233/41,000) of the excreted D is derived from the peripheral conversion of 17α-hydroxypregnenolone. This finding agrees with the calculation made by multiplying the secretory rate of 17α-hydroxypregnenolone (63 mg.) by the observed percentage conversion of this precursor to D (0.9 per cent). The amount of the product, 0.57 mg., is about 0.5 per cent of the total daily excretion of 110 mg. This conversion is of the same order of magnitude as

that (2.8 per cent) observed by Solomon *et al.* (7) in a patient with extensive metastases arising from an adrenal tumor. In this latter instance, some of the transformation of the labeled C_{21} steroid to the C_{19} metabolite probably occurred as a result of enzymes present within the tumor tissue. In the situation reported in this paper, it is also possible, but unlikely, that a small fraction of the labeled precursor may have reached the adenomatous adrenal and was similarly metabolized by it. If this were so, the peripheral, *extra-adrenal* conversion of 0.5 per cent would have been overestimated, but the actual contribution of the endogenously secreted 17α-hydroxypregnenolone to urinary D would have been correctly estimated.

Obviously then, an adrenal secretory product other than 17α-hydroxypregnenolone must have been the precursor of the bulk of the urinary DHS. The results described in this paper provide no direct information concerning the identity of this precursor. However, the likely possibilities are limited, since all precedents would appear to require that the precursor of D be also a Δ^5-3β-hydroxy steroid. There is no evidence that a urinary metabolite having this structural feature can arise from a steroidal precursor which lacks it. The following Δ^5-3β-hydroxy steroids have been isolated either from adrenal tissue or from adrenal venous blood: dehydroisoandrosterone (1), Δ^5-pregnenolone (20), 17α-hydroxypregnenolone (5), and Δ^5-androstene-3β,17β-diol (3). Although it has been claimed (6) that, in the dog, pregnenolone can be converted by extra-adrenal tissue to dehydroisoandrosterone, Polley and Mason (21) and McGavack *et al.* (22) were unable to find any increase in the levels of the urinary 17-ketosteroids even after the administration of enormous amounts of pregnenolone to human subjects. Moreover, the peripheral conversion of a C_{21}-17-deoxy steroid to a C_{19} steroid has not been demonstrated in the human. Hirschmann *et al.* (3) eliminated Δ^5-androstenediol from consideration as an important precursor of urinary D. Since we have already shown that 17α-hydroxypregnenolone was not the precursor of urinary D in this patient, the only remaining contender for the precursor of urinary D is D secreted by the adrenal. Since the patient insisted upon immediate surgery, we were unfortunately unable to prove this directly by studying the metabolism of D in her case.

In normal subjects, the conversion of 17α-hydroxypregnenolone to urinary D is even smaller (7) than the conversion observed in this patient with adrenal adenoma. For this reason, and for those already advanced (12), it is reasonable to conclude that the precursor of the urinary D found in normal urine is likewise D secreted by the adrenal. The failure to isolate D in some samples of adrenal vein blood (2) could well be explained by the fact that these blood samples were drawn from elderly patients with advanced metastatic cancer. It is known that such patients excrete only a

small amount of 11-deoxy-17-ketosteroids (23) in the urine, from which it can be inferred that the amount of D synthesized by them is also small.

Recently, Baulieu (24) presented evidence that tumors of the adrenal can secrete DHS. In addition to isolating the sulfate from such a tumor, he also demonstrated that the concentration of the conjugate was greater in the adrenal venous effluent than it was in the peripheral plasma. Thus, DHS synthesized by the adrenal could also be a precursor of urinary DHS. Since D and DHS have been shown to be interconvertible *in vivo* (25), the biologic consequences of either being produced by the adrenal might be identical.

Of the injected radioactivity, 0.24 per cent was recovered as androsterone and 2.8 per cent as etiocholanolone. The urinary excretions of these 17-ketosteroids were 7 and 20 mg. per day respectively. The preponderance of radioactivity present in the etiocholanolone fraction as compared to that found in the androsterone fraction, is consistent with the findings of previous studies (26) which demonstrated that when Δ^4-3-ketosteroids possessing 17α-hydroxy-20-keto side-chains are metabolized to 17-ketosteroids, the isomer formed in largest amount belongs to the 5β series. From the data on percentage conversion, it is evident that the 63 mg. of 17α-hydroxypregnenolone produced daily by this patient would contribute only 0.1 mg. to urinary androsterone and 1.7 mg. to urinary etiocholanolone, *i.e.*, only a small fraction of the amounts of these 17-ketosteroids actually excreted. Since the endogenous production of 17α-hydroxyprogesterone has been shown to be negligible, it would appear that most of these urinary 17-ketosteroids were derived from a C_{19} precursor such as dehydroisoandrosterone, Δ^4-androstenedione or testosterone. Our data do not permit a direct determination of that fraction of the urinary androsterone and etiocholanolone that was derived from D and that which was derived from androstenedione and/or testosterone. The principal precursor of the urinary D must have been secreted in amounts at least equal to the 110 mg. found in the urine. Since this precursor undoubtedly also contributed to the urinary A and E, the contributions from androstenedione and/or testosterone must have been relatively small. Both androstenedione and testosterone are efficiently converted into urinary A and E, and since the total concentration of these metabolites in urine was only 30 mg. per day, it is reasonable to conclude that the amount of androstenedione and/or testosterone produced in this patient must have been small. Nevertheless, in view of the great differences in the biologic activities of the Δ^4-3 ketones as compared with that of D, small amounts of these compounds may have accounted for the virilization observed in this patient.

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BILIARY AND URINARY METABOLITES OF 4-C^{14}-17α-HYDROXYPROGESTERONE IN HUMAN SUBJECTS*

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ABSTRACT

The metabolism of 4-C^{14}-17α-hydroxyprogesterone, injected intravenously into 6 endocrinologically normal women (3 with bile fistulas), was essentially like that described previously by other investigators for large quantities of 17α-hydroxyprogesterone. Differences from reported results were the presence of tetrahydrocortisone, the absence of significant amounts of androgenic 17-ketosteroids, and the predominance of pregnane-3α,17α-diol-20-one over pregnanetriol in the urine. The biliary metabolites, reported here for the first time, were similar to those in the urine. The endogenous daily production of 17α-hydroxyprogesterone was calculated to be approximately 2 mg.

17α-Hydroxyprogesterone is an important intermediate in the biosynthesis of steroid hormones. It is a precursor of cortisol (1) and androstenedione (2), the latter itself being a precursor of testosterone and estrogens. Since in most forms of the adrenogenital syndrome, 17α-hydroxyprogesterone appears to be a major steroid secreted by the adrenal cortex (3), knowledge of its metabolism is of considerable biochemical and clinical interest. Even though several papers (4–9) have been published on this subject, quantitative aspects of 17α-hydroxyprogesterone metabolism are largely unknown. This may be due to the use in the past of unlabeled 17α-hydroxyprogesterone. Recently, moreover, it has been shown that after the intravenous injection of 4-C^{14}-17α-hydroxyprogesterone into human subjects with bile fistulas, an appreciable amount of radioactivity is excreted in the bile (10). Identification of these biliary metabolites has not, to our knowledge, been accomplished. In this publication, therefore, we wish to report an analysis of urinary and biliary excretory metabolites following the injection of 4-C^{14}-17α-hydroxyprogesterone into human subjects.

PROCEDURE

Clinical material

Urine and bile samples were obtained from 6 women who, from an endocrinologic point of view, were considered to be normal. Three of these women had bile fistulas

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after cholecystectomy for cholelithiasis. Bile and urine were collected separately. Patients received from 2.0 to 4.5 μc. of 4-C^{14}-17α-hydroxyprogesterone (40 μc. per mg.) by the intravenous route. One subject received an injection of 100 mg. of carrier 17α-hydroxyprogesterone, and at the end of the infusion the C^{14}-labeled steroid was administered through the tube. Approximately 99 per cent of the radioactive steroid obtained from the New England Nuclear Corporation possessed the same mobility as standard 17α-hydroxyprogesterone upon paper chromatography. The 1 per cent impurity was less polar than 17α-hydroxyprogesterone. Details concerning the clinical material may be found elsewhere (10).

Details concerning the collection of urine and bile, the hydrolysis and extraction of the steroids, and the measurement of radioactivity have been described elsewhere (10). Briefly, they consisted of extraction of unconjugated steroids followed by enzymatic hydrolysis (Ketodase) and continuous extraction at pH 1 for forty-eight hours. In this way, 40–60 per cent of the administered radioactivity could be recovered. Extracts of urine from patients without a bile fistula were pooled (Extract NBF), as were the extracts of urine from the bile-fistula patients (Extract BF). The extract of urine (Extract C) from the one subject who received, in addition to the tracer, 100 mg. of carrier intravenously was processed separately. All extracts of bile were also pooled (Extract B). The extracts resulting from strong acid hydrolysis were not included in the study because of possible effects produced by the procedure.

Nonradioactive steroids

Pregnane-3α-ol-20-one, pregnane-3β-ol-20-one, 5α-pregnane-3α-ol-20-one and 5α-pregnane-3β-ol-20-one, which served as starting materials for the synthesis of 17-hydroxy-20-ketosteroids, as well as pregnane-3α,17α-diol-20-one, 5α-pregnane-3β,17α-diol-20-one, etiocholan-3α-ol-17-one and etiocholane-3,17-dione, were obtained from the Elite Chemical Company.[1]

Pregnane-3β,17αdiol-20-one and 5α-pregnane-3α,17α-diol-20-one were synthesized from pregnane-3β-ol-20-one and 5α-pregnane-3α-ol-20-one, respectively, by the method of Kritchevsky and Gallagher (11). The intermediary 17:20-enol acetates, however, were obtained by the use of isopropenyl acetate instead of acetic anhydride.

Pregnane-17α-ol-3,20-dione and 5α-pregnane-17α-ol-3,20-dione were synthesized by N-bromoacetamide oxidation of pregnane-3α,17α-diol-20-one and 5α-pregnane-3β,17α-diol-20-one, respectively (12).

Pregnane-3α,17α,20β-triol, pregnane-3β,17α,20β-triol, 5α-pregnane-3α,17α,20β-triol and 5α-pregnane-3β,17α,20β-triol were obtained by sodium borohydride reduction of the corresponding pregnane-3,17α-diol-20-one as described by Fukushima and Meyer (13). In the reaction mixture the ratio of 20β- to 20α-triols was 10:1–20:1. Purification was performed by gradient elution chromatography on alumina.

Pregnane-3α,17α,20α-triol, pregnane-3β,17α,20α-triol, 5α-pregnane-3α,17α,20α-triol, and 5α-pregnane-3β,17α,20α-triol were synthesized by lithium aluminum hydride reduction of the corresponding 17α,20β-epoxy-5α(or β)-pregnane-3,20α-diol diacetates (13), obtained from the corresponding pregnane-3-ol-20-ones by epoxidation with perbenzoic acid of their 17:20-enolacetates (11).

All nonradioactive standards were crystallized until sharp melting points in agreement with those published were obtained.

Countercurrent distribution (CCD)

The urine and bile extracts dissolved in ether were first washed with 0.1 *N* NaOH and

[1] Address: 23 Elm Street, Newark 2, New Jersey.

TABLE 1. COUNTERCURRENT DISTRIBUTION OF EXTRACTS IN THE SYSTEM CYCLOHEXANE:ETHYL ACETATE/METHANOL:WATER, 600:400/600:400 (n = 100)

Extract		Fraction			
		I	II	III	IV
BF	Tube No.	0–23	24–48	49–71	72–99
	%*	4.8	30.1	59.5	6.2
NBF	Tube No.	0–25	26–47	48–70	71–99
	%	8.7	31.6	51.1	8.6
C	Tube No.	0–22	23–45	46–70	71–99
	%	4.5	36.7	50.9	7.9
B	Tube No.	0–26	27–50	51–99	
	%	7.1	36.7	55.2	

* Per cent of total radioactivity in extract.

water and evaporated to dryness. Each extract was separated into 4 fractions (Table 1) by CCD. Each fraction was subsequently separated into its ketonic and nonketonic moiety by Girard fractionation. Since it was found that the presence of nonketonic material in fraction III was attributable to overlapping of fractions II and III in the CCD, the nonketonic portions of fractions II and III were pooled (Nonketonic Fraction II (+III)). For the same reason the ketonic fractions II and III were also pooled (Ketonic III (+II)). Subsequent separation was accomplished by column chromatography.

Silica gel chromatography

This was used as a means of purification of countercurrent distribution fraction I. Six Gm. of silica gel suspended as a slurry in chloroform was poured into a column (1×25 cm.) with a fritted glass plate for support. The extract, dissolved in chloroform, was absorbed onto the column and was eluted with a gradient elution system of pure chloroform in the receiving vessel and pure acetone in the donor (affluent ratio 1:1). The flow was unrestricted. Radioactivity contained in fractions 10–25 (10 ml. each) was pooled.

Alumina chromatography

Alumina chromatography was performed by the gradient elution technique (14) using 15 Gm. of neutral alumina (Woelm[2] Grade I) (Table 2).

Paper chromatography

In order to check the purity of different fractions from the column, several conventional (15, 16) paper chromatographic systems were used: A) Skellysolve C/85% methanol; B) benzene:Skellysolve C/methanol:water, 33:67/80:20; C) hexane/formamide; D) toluene/propylene glycol; and E) toluene:n-heptane/methanol:water, 75:25/80:20.

[2] Alupharm Chemicals, P.O. Box 755, New Orleans, Louisiana.

TABLE 2. SYSTEMS USED IN GRADIENT ELUTION CHROMATOGRAPHY

System No.	Fraction No.*	Water content of alumina, %	Volume of receiver (ml.)	Composition of receiver†	Composition of donor†	Ratio effluent receiver/donor
II	II	2	600	0.5	10	5:1
III	III	4	600	0.25	6	5:1
IV	IV	4	700	0.0	4	7:1

* For identification of fractions, see Table 1.
† Expressed as per cent methanol in benzene.

Identification

Identification was based on the reverse isotopic dilution technique. After 10–20 mg. of carrier had been added to an isolated radioactive metabolite, the carrier was recrystallized until the specific activity remained constant for at least two successive crystallizations. In the study in which 100 mg. of 17α-hydroxyprogesterone was also given (Extract C), infrared spectrophotometry, recrystallization to constant specific activity and melting points were used for identification.

RESULTS

CCD Fraction I

After purification of the ketonic fraction on silica gel, the extract was chromatographed in the toluene/propylene-glycol system for seventy-two hours and afforded 2 radioactive peaks with a mobility similar to that of tetrahydrocortisone and of tetrahydro compound S or 11-ketopregnanetriol, respectively. Since the less polar peak represented only a very small amount of radioactivity, the compound was not studied further. Crystallization of the more polar compound afforded 2 mg. of material with a specific activity of 1820 counts/minute/μmole and an infrared spectrum identical to that of tetrahydrocortisone. Reverse isotopic dilution of the compound with pregnane-3α,17α,21-triol-11,20-dione afforded crystals with a constant specific activity which did not change upon formation of its dinitrophenylhydrazone. The nonketonic portion of Fraction I afforded only one peak upon chromatography in system II, and this was identified as pregnane-3α,17α,20α-triol.

Nonketonic Fraction II (+III)

Chromatography on alumina afforded 2 well defined peaks and a less polar plateau. The most polar material, accounting for about 90 per cent of the radioactivity applied to the column, was pregnane-3α,17α,20α-triol. Slightly less polar, and accounting for about 5 per cent of the fraction, was pregnane-3α,17α,20β-triol. In Extract C, prior to chromatography, this fraction was separated by digitonin precipitation into its 3α and 3β components. The radioactivity was nearly exclusively (98.5 per cent) in the 3α portion; moreover, subsequent analysis showed that most of the radio-

activity in the 3β fraction was attributable to pregnane-3α,17α,20α-triol. Besides the aforementioned two 5β-pregnanetriols, alumina chromatography of the 3α portion of Extract C furnished a small amount of a less polar compound which, upon reverse isotopic dilution, proved to be 5α-pregnane-3α,17α,20α-triol. In all experiments very small amounts of nonpolar steroids were found which could not be identified with certainty.

Ketonic Fraction III (+II)

Chromatography on alumina afforded 2 fractions, the most polar of which accounted for 80–95 per cent of the radioactivity in this fraction and was shown to be pregnane-3α,17α-diol-20-one. The less polar fraction was further resolved on alumina chromatography in the system used for Fraction IV, into 2 components. The more polar, accounting for 60–75 per cent of this fraction, was pregnane-17α-ol-3,20-dione; the less polar, 5α-pregnane-17α-ol-3,20-dione.

Fraction IV

Since the nonketonic portion of Fraction IV represented only about 1 per cent of the total urinary extract, this fraction was not studied further.

The ketonic portion was chromatographed in System IV and separated into 3 ill-defined peaks. Since chromatography of pure steroids had shown us previously that mixtures of pregnane-17α-ol-3,20-dione, androsterone, 5α-pregnane-17α-ol-3,20-dione and etiocholanolone would be incompletely resolved, the 3 peaks were recombined and the material was acetylated with pyridine and acetic anhydride before rechromatography on alumina using a modified System IV. The acetylated material was applied to the column in benzene-petroleum ether (1:1) and developed with 100 ml. of the same solvent. Afterwards, chromatography was continued with System IV. This afforded 3 peaks of radioactivity; the least polar peak was eluted with benzene-petroleum ether and was identified as etiocholanolone acetate. The other 2 peaks were identified as 5α-pregnane-17α-ol-3,20-dione and pregnane-17α-ol-3,20-dione, respectively.

Table 3 summarizes the metabolites which were identified and the percentage of the urinary extracts which they represented.

DISCUSSION

As far as the qualitative aspects of 17α-hydroxyprogesterone metabolism are concerned, our results largely confirm those obtained previously (4, 7–9), *i.e.*, 17α-hydroxyprogesterone is largely metabolized to pregnanetriol and pregnanediolone. 5α-Pregnane-17α-ol-3,20-dione and etiocholanolone are minor metabolites.

No pregnanolones were observed in the urine and bile specimens ana-

lyzed. Pregnanolones have been shown by Fukushima and Gallagher (17) to be artifacts formed by the pinacol-pinacolone rearrangement of 17:20-glycols induced by mineral acid hydrolysis. In our experiments, solvolysis (18) was performed under much milder conditions and this may have prevented formation of these artifacts.

Rather surprising, however, was the finding of radioactive tetrahydrocortisone. 17α-Hydroxyprogesterone is known to be a precursor of cortisol in the adrenal (1). Although the radioactivity attributable to tetrahydrocortisone represented only a minute percentage of the radioactivity injected, it seems more than could be expected from adrenal perfusion.

It is of interest that in all the extracts studied, pregnane-3α,17α-diol-20-one was quantitatively by far the most important metabolite, representing about 40 per cent of the radioactivity present in the total extract or 25 per cent of the administered radioactivity. This differs from the results of Brooks and Prunty (9) and of Axelrod and Goldzieher (8), who found pregnanetriol to be predominant over pregnanediolone. There are some differences in technique and choice of subjects which might account for this discrepancy. The latter investigators used only hydrolysis with β-glucuronidase (Ketodase) and administered 17α-hydroxyprogesterone orally; the former studied patients with the adrenogenital syndrome. Although Axelrod and Goldzieher (8) isolated 5α-pregnane-3β,17α-diol-20-one and 5α-pregnane-3α,17α-diol-20-one from the urine of their patients, we were unable to detect these metabolites. If present, these compounds would represent less than 1 per cent of the radioactivity in the extract.

TABLE 3. METABOLITES ISOLATED FROM URINE AND BILE AFTER INTRAVENOUS ADMINISTRATION OF 4-C^{14}-17α-HYDROXYPROGESTERONE

(expressed as per cent of radioactivity present in the total extract)

Metabolite identified	Extract			
	BF	NBF	C	B
Pregnane-3α,17α,20α-triol	26.0	30.2	29.8	25.9
Pregnane-3α,17α,20β-triol	3.4	1.7	2.7	—
5α-Pregnane-3α,17α,20α-triol	—	—	1.2	—
Pregnane-3α,17α-diol-20-one	53.0	38.9	34.5	38.5
Pregnane-17α-ol-3,20-dione	0.5	0.3	0.2	1.7
5α-Pregnane-17α-ol-3,20-dione	0.3	0.1	0.1	3.0
Etiocholan-3α-ol-17-one	—	0.3	0.1	—
Pregnane-3α,17α,21-triol-11,20-dione	0.3		0·2	—
Total	83.3	71.7	68.8	69.1

Pregnane-3α,17α,20α-triol accounted for 25–30 per cent of the radioactivity in the extract, whereas pregnane-3α,17α,20β-triol, the only other pregnanetriol regularly found, accounted for about 2.5 per cent. In only one instance (C) could we identify 5α-pregnane-3α,17α,20α-triol.

Of the 17-ketosteroids, only etiocholanolone could be identified and it accounted for less than 0.5 per cent of the radioactivity. No androsterone, androstenedione, or etiocholanedione was found. If present, these compounds would appear to represent a very small fraction of the radioactivity. The identification of etiocholanolone as the most important 17-ketosteroid derived from the administered 17α-hydroxyprogesterone suggests that cleavage of the side-chain of 17α-hydroxyprogesterone occurs after saturation of Ring A, thus producing largely compounds of the 5β series. It has been suggested by Jailer *et al.* (4) that virilism in the adrenogenital syndrome is induced by the piling up of partially hydroxylated hydrocortisone precursors, mainly 17α-hydroxyprogesterone, which escape into the circulation and are metabolized along well known pathways, resulting in the production of androgens. Our results do not support this hypothesis, as only a minimal percentage of 17α-hydroxyprogesterone was metabolized to 17-ketosteroids. This suggests that masculinization in the adrenogenital syndrome results from androgens synthesized in the adrenals.

Metabolites in bile were essentially similar to those in urine. Moreover, the bile fistula did not seem to affect the quantitative or qualitative urinary excretion of 17α-hydroxyprogesterone metabolites. This suggests that an enterohepatic circulation of 17-hydroxyprogesterone metabolites probably does not exist, but that, once excreted in the bile, the metabolites are primarily eliminated in the feces. This is similar to the metabolism of progesterone (19), but differs completely from that of the estrogens (20). Previous studies have shown that following progesterone administration the bile tends to contain relatively less of the more polar metabolites than does the urine (21). This could not be demonstrated for 17α-hydroxyprogesterone. The only difference between the steroid pattern in urine and bile was the significantly higher percentage in bile of the pregnane-17α-ol-3,20-diones.

From the data presented, the amount of 17α-hydroxyprogesterone produced in the body daily can be calculated. The calculations are based on the assumption that the sole origin of pregnanetriol is 17α-hydroxyprogesterone. To our knowledge, no conflicting evidence has been published. Two of the subjects without bile fistulas received an injection of radioactive 17α-hydroxyprogesterone having a specific activity of 40,000 counts/minute/μg. The pregnanetriol isolated from the urine (collected for seventy-two hours) of these subjects revealed a specific activity of 600 counts/minute/μg. This indicates a 67-fold dilution of the isotope, corresponding to a production of 17α-hydroxyprogesterone of 1.1 mg. per twenty-four hours per subject. In 3 subjects with bile fistulas the calculations revealed a daily production of 1.65 mg. per subject. These figures are in close agreement with those published for the daily urinary pregnanetriol excretion ($<$2.0 mg.) in subjects without endocrine abnormalities (22, 23).

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Addendum

In a recent report, published since the completion of the present paper, Fukushima *et al.* (24) have described the metabolism of C^{14}-17α-hydroxyprogesterone in 1 endocrinologically normal male subject and in 1 patient with congenital adrenal hyperplasia. In general, the urinary metabolites isolated from the urines of these 2 subjects were very similar to those reported in the present paper, with the exception of the finding of androsterone by Fukushima *et al.* (24). It should be pointed out, however, that the total amount of androsterone and etiocholanolone excreted in the urine constituted a very small percentage of the injected radioactive 17α-hydroxyprogesterone.

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FAILURE OF VASOPRESSIN OR OXYTOCIN TO STIMULATE THYROID FUNCTION IN THE NORMAL MALE

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ABSTRACT

In a group of normal adult males the administration of 0.2–0.4 I.U. per Kg. of vasopressin (5 subjects) or 0.4 I.U. per Kg. of oxytocin (5 subjects) intravenously four days after an oral tracer dose of radioiodine caused no significant increase in the plasma level of the radioactive protein-bound iodine nor decrease in the thyroidal radioiodine content. In 3 additional men, the parameters used were significantly altered by 10 I.U. of thyrotropin given intramuscularly. Therefore, under the conditions studied, neither vasopression nor oxytocin stimulate the human thyroid, either directly or by way of thyrotropin release.

THE secretion of tropic hormones by the adenohypophysis has been demonstrated to be under the influence of the hypothalamus. Observations have been made which suggest that one or more hypothalamic neurohumoral agents secreted by the median eminence into the hypophyseal portal system effect release of the pituitary hormones (1). The precise nature of these transmitting materials is unknown. Guillemin *et al.* (2), believe that the neurohumoral substance that causes release of adrenocorticotropin (ACTH) may be one or more peptides related to vasopressin or oxytocin. Other evidence has been interpreted as indicating that vasopressin and oxytocin may effect an increased release of anterior pituitary hormones, including ACTH, gonadotropin and thyroid-stimulating hormone (TSH) (3–7). Harris (8) has demonstrated release of protein-bound I^{131} from the rabbit thyroid *in vivo* following administration of vasopressin. Other authors (9, 10) have reported no effect of vasopressin and oxytocin upon TSH release. Because of these varied observations, the present study of the effects of vasopressin and oxytocin upon thyroid function in the human was undertaken.

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* Veterans Administration Clinical Investigator.

MATERIAL AND METHODS

The studies were carried out on 19 hospitalized male patients under the age of 45 who had no clinical or laboratory evidence of endocrine disease. They were divided into four groups. One group of 6 subjects served as controls; a second group of 3 patients received 10 units of thyrotropin (Thytropar, Armour) intramuscularly. The third group was composed of 5 patients who received synthetic oxytocin (Syntocinon, Sandoz), 0.4 I.U. per Kg. of body weight intravenously in a two-hour infusion. The fourth group of 5 subjects was given vasopressin (Pitressin, Parke-Davis) in doses ranging from 0.2 to 0.4 I.U. per Kg. of body weight intravenously during a two-hour period. The higher dose could not be administered to 2 of the subjects because of severe nausea and abdominal cramps, but in no case did any patient receive less than a total of 0.2 I.U. per Kg. of body weight. The subjects were not used as their own controls, in an effort to minimize exposure to radiation.

The substances under study were injected ninety-six hours after the administration of an oral tracer dose of between 100 and 150 μc. of carrier-free sodium iodide^{131}. A standard was retained for reference counting. Measurements of radioactivity over the thyroid gland were made periodically with a lead-shielded scintillation counter. Samples of heparinized blood were drawn at specific intervals and processed by the ion-exchange column method (PBI-Rezikit, Squibb). The radioactivity in the plasma effluent was determined in well-type scintillation counters and the plasma radioactive protein-bound iodine concentration (PBI^{131}) was calculated in terms of the percentage of the administered dose per liter of plasma present in each specimen. Blood samples for protein-bound iodine concentration (PBI) were obtained from some patients either twenty-four or forty-eight hours before, and again twenty-four hours after injection of vasopressin, oxytocin or TSH. The PBI determinations were performed by the Bio-Science Laboratories by a modification of the method of Barker *et al.* (11).

RESULTS

The data in Figures 1 and 2, and in Table 1 indicate that vasopressin, given intravenously in quantities between 0.2 and 0.4 I.U. per Kg., failed to increase plasma PBI^{131} concentration or alter thyroidal radioactivity. A similar lack of response in these parameters was noted in the group that received 0.4 I.U. per Kg. of oxytocin. In the subjects that were given TSH, there was a significant increase in the plasma PBI^{131} level and a significant decrease in thyroidal radioactivity.

The infusion of oxytocin produced no change in mean serum PBI concentration, whereas in the group of subjects that received TSH there was

TABLE 1. MEAN *percentage change* IN INDICES OF THYROID FUNCTION IN THE 24-HOUR PERIOD FOLLOWING THE ADMINISTRATION OF THE EXPERIMENTAL SUBSTANCE WHICH WAS GIVEN FOUR DAYS AFTER A TRACER DOSE OF RADIOIODINE

	Control		Thyrotropin			Oxytocin			Vasopressin		
	Mean	SD	Mean	SD	P*	Mean	SD	P*	Mean	SD	P*
PBI^{131}	+25.4	±22.2	+194.1	±106.1	<.01	+22.4	±7.8	n.s.**	+20.6	±12.6	n.s.
Thyroidal radioiodine retention	+ 0.2	± 2.0	− 11.0	± 4.0	<.001	− 3.5	±5.8	n.s.	+ 1.5	± 8.9	n.s.

* P refers to comparison with control group.
** Not significant (P =0.3 or greater).

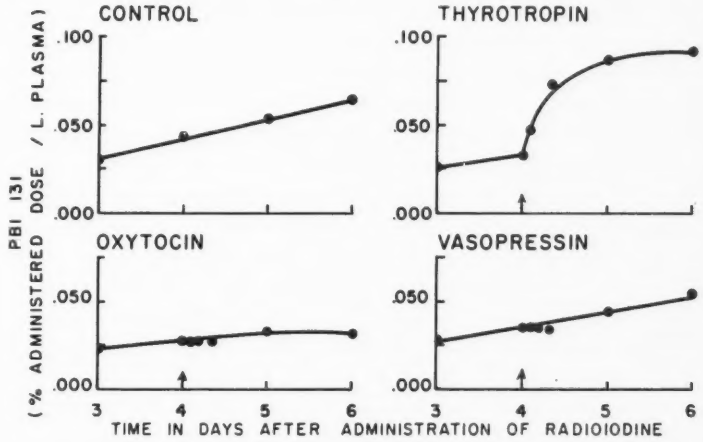

FIG. 1. Mean plasma protein-bound radioiodine concentration *vs.* time, in control subjects and subjects given oxytocin, vasopressin or TSH at the times indicated by the arrows.

an increase in the mean serum PBI level from 4.5 μg. per 100 ml. before stimulation to 7.9 μg. per 100 ml. twenty-four hours after administration. In the group that received vasopressin, there was an insufficient number of serum PBI determinations for analysis of the effects.

DISCUSSION

It would appear from these data that large intravenous doses of vasopressin and oxytocin fail to stimulate thyroid function in normal men as measured by parameters that are significantly altered by 10 I.U. of TSH

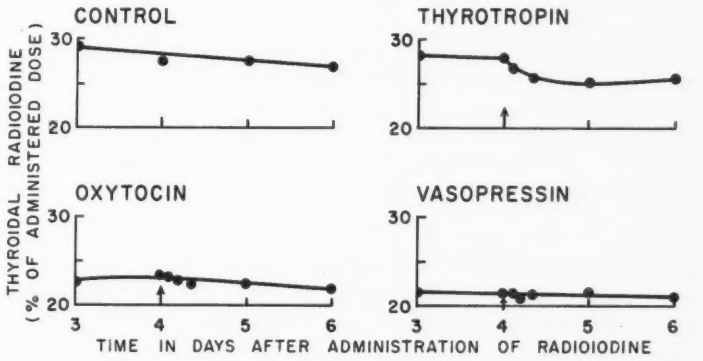

FIG. 2. Mean thyroidal radioiodine retention *vs.* time, in control subjects and subjects given oxytocin, vasopressin or TSH at the times indicated by the arrows.

given intramuscularly. It has been reported that neurohypophyseal hormones fail to affect thyroid function as measured by thyroid hormone secretion in rats (9) and in cats and rabbits (12), but Harris (8) has observed an increased thyroidal release of PBI^{131} in rats after administration of vasopressin. Various concentration of posterior pituitary extracts perfused directly and continuously into the grafted or intact pituitary of animals have not been found to stimulate TSH release (10). On the other hand, Fraja and Martini (3) have reported that the intravenous administration of posterior pituitary hormones, particularly oxytocin, causes the liberation of TSH from the pituitary of the dog. Bottari (7) has demonstrated an increase in circulating TSH levels at one, two, and three hours following the intraperitoneal administration of 0.2 I.U. per Kg. of vasopressin in the rabbit. Some of these discrepancies in response might be due to species differences, or to an effect of vasopressin on the thyroid or on the peripheral utilization of thyroid hormone that would mask the response to an increased level of TSH. It is conceivable that the octapeptides, given intravenously, do not reach a concentration in the hypophyseal portal system of the human being that is adequate for TSH release. However, the experiments of Fraja and Martini (3) and of Bottari (7) would indicate that in the dog and rabbit the systemic administration of quantities of vasopressin and oxytocin in the range used in our study does increase TSH release.

Einhorn (13) has demonstrated that there is a direct relationship between plasma PBI^{131} concentration and increments in PBI^{131} following the administration of TSH. Therefore, when PBI^{131} changes are expressed as a percentage of the values obtained immediately preceding the injection of TSH, there is less variability than when PBI^{131} increments are expressed as absolute values. Mertz (14) has noted that the administration of Pitressin tannate in oil to human subjects, some of whom were hyperthyroid and some euthyroid, produced an increase in thyroid function as measured by an absolute increase in the level of circulating PBI^{131}. However, if the data from his euthyroid patients are analyzed after excluding the values for thyrotoxic patients (in whom small relative variations would be reflected in large absolute differences) there is no significant increase in PBI^{131} concentration following administration of vasopressin ($P = 0.4$).

It is of interest that following administration of TSH, the percentage increment of PBI^{131} is greater than that of the PBI. This confirms the observations of Einhorn (13) that the PBI^{131} level, under the conditions studied, is a more sensitive indicator of TSH effect than is the chemically determined PBI level. The marked change caused by TSH in plasma PBI^{131} concentration in comparison with the significant but lesser percentage change in thyroidal radioactivity would appear to be a reflection of the difference in the intrathyroidal and extrathyroidal organic iodine pools that

Berson and Yalow (15) have described. Our observation that the concentration of PBI^{131} continued to rise throughout the six days of the control studies agrees with data previously reported by Berson and Yalow (15) and Becker *et al.* (16), but does not agree with the report of Quimby *et al.* (17) that the plasma PBI^{131} concentration in euthyroid subjects reaches a maximum seventy-two hours after administration of the radioiodine.

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THE PERIPHERAL METABOLISM OF TRI- AND TETRAIODOTHYROACETIC ACIDS IN MAN*†

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ABSTRACT

The metabolism of tracer doses of I^{131}-labeled 3,5,3′-triiodothyroacetic acid (Triac) and tetraiodothyroacetic acid (Tetrac) was studied in euthyroid subjects and in patients with partial external bile fistulas. In normal subjects, Triac was very rapidly cleared from the blood and concentrated by the liver; both Triac and Triac glucuronide were excreted in high concentrations in the bile. Most of the administered radioactivity rapidly appeared in the urine as inorganic I^{131}. Tetrac was metabolized quite differently. It disappeared from serum more slowly than Triac; an apparent mean half-time in serum of 3.3 days was observed. Tetrac was less highly concentrated in the liver and in the bile. Most of the administered radioactivity ultimately appeared in the urine as inorganic iodide. Formation of conjugates, which appeared to be an important pathway in the metabolism of Triac, was less prominent in the case of Tetrac. These findings are compared with prior studies of the peripheral metabolism of *l*–3,5,3′-triiodothyronine and *l*-thyroxine, and are considered in the light of previous observations concerning the binding of these several compounds to plasma proteins.

THE peripheral metabolism of *l*-thyroxine (T_4) and of *l*–3,5,3′-triiodothyronine (T_3), the two major hormones secreted by the thyroid gland, has been extensively studied in man (1–6). Correlative studies have shown that, when eumetabolic status is maintained, the fractional rate of turnover

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of thyroxine peripherally is often inversely related to the binding capacity of the thyroxine-binding globulin (TBG) in serum (7–10). Therefore, it seems likely that the binding of T_4 to TBG is an important determinant of the rate at which T_4 is metabolized in man (*cf.* reviews, references 11–13). However, the physiologic role of the more recently described thyroxine-binding pre-albumin (TBPA) (14) is less clearly defined.

Recently, electrophoretic analyses have indicated that several derivatives of T_4 and T_3 have binding properties which differ greatly from those of the parent compounds. Of particular interest was the finding that deamination induces or enhances binding by TBPA in human serum, and abolishes binding by TBG (15). In view of this predominant binding of the deminated derivatives to TBPA, it appeared possible that the peripheral metabolism of these derivatives might differ from that of T_4 and T_3 in a manner which could be correlated with their known differences in protein binding. In turn, reported peculiarities in their biologic activity (16) might be explained by unusual features in their peripheral metabolism.

The metabolism of labeled tracer quantities of several deaminated derivatives has been studied in laboratory animals (17–22). However, since the binding of these derivatives by the serum proteins of these species is unknown, the animal studies do not allow correlations between binding and metabolism to be made. Except for a study of the disappearance of labeled triiodothyropropionic acid from the serum of one patient (16), no such studies have been made in man.

Thus, studies were undertaken of the peripheral metabolism in man of I^{131}-labeled tri- and tetraiodothyroacetic acids (Triac and Tetrac), compounds of particular interest since they have been shown to be naturally-occurring products of the peripheral metabolism of T_4 and T_3 (23).

MATERIALS AND METHODS

Materials

Triac and Tetrac, labeled with I^{131} in the phenolic ring, were obtained from a commerical source.[1] The initial specific radioactivity of both varied from batch to batch, but usually approximated 30 mc. per mg. When shipped by the supplier, compounds were 95–98 per cent pure, as assessed chromatographically, and inorganic iodide was the sole contaminant. Immediately upon receipt, compounds were diluted to a concentration of 30 μc. per ml. in 1 per cent human serum albumin, and these solutions were used for injection into patients, usually within forty-eight hours after their preparation. At the time of use, purity was re-assessed by ascending filter-paper chromatography in butanol-dioxane-2*N* ammonia (4:1:5). The latter studies revealed that injection solutions of Triac were chromatographically homogeneous to the extent of 85–95 per cent; the remainder was chiefly iodide, with occasional traces of material at the solvent front. On more prolonged standing, subsequent to use for intravenous injection, two unknown

[1] Abbott Laboratories, Oak Ridge, Tennessee.

TABLE 1. CLINICAL FEATURES OF PATIENTS IN WHOM THE PERIPHERAL METABOLISM OF TRIAC AND TETRAC WAS DETERMINED

Patient, age & sex	Diagnosis	Compound studied
E.D.—50 F	Anxiety neurosis	Triac
W.L.—44 F	Nontoxic nodular goiter	Triac
M.W.—49 F	Graves' disease, post-I^{131} myxedema; receiving desicc. thyroid, 2 gr. daily	Triac
H.K.—52 M	Scalp laceration; chronic alcoholism	Triac
H.G.—60 M	Arteriosclerotic heart disease	Triac
H.T.—72 M	Chronic alcoholism	Triac
J.L.—56 M	Chronic alcoholism	Triac
A.R.—57 F	Primary myxedema; receiving desicc. thyroid, 2 gr. daily	Tetrac
W.S.—40 M	Alcoholic gastritis; pulmonary tuberculosis, arrested	Tetrac
J.K.—63 M	Arteriosclerotic heart disease; diabetes mellitus	Tetrac
A.W.—27 M	Mental retardation	Tetrac
R.R.—35 M	Diffuse toxic goiter, untreated	Tetrac
F.W.—40 M	Chronic alcoholism	Tetrac

compounds appeared between iodide and Triac. Tetrac was 90–95 per cent pure, and the remainder was mainly iodide with occasional traces (always less than 1 per cent) of front-running material and of material with the chromatographic mobility of Triac.

Subjects

For the major portion of these studies, patients were selected from the wards or Out-Patient Department of the Boston City Hospital, and were admitted to a metabolic ward. Pertinent clinical information concerning these patients is recorded in Table 1. Except for one severely thyrotoxic patient, all were clinically well, and were judged to be euthyroid by clinical criteria, serum protein-bound iodine (PBI) values and, when indicated, determinations of thyroidal radioiodine uptake and basal metabolic rate.

Studies of biliary excretion of the derivatives were carried out on surgical wards, in patients convalescing from biliary-tract surgery who had partial external bile drainage through a T-tube. Clinical data on these patients are presented in Table 2.

Methods

Clinical. The standard method of study was similar to that used in previous studies of the peripheral turnover of labeled T_4 (4, 6). With the exception of the 2 subjects (M.W. and A.R.) receiving desiccated thyroid for treatment of myxedema, all patients received methimazole, 30 mg. every six hours, a dose sufficient to block organification of iodine by the thyroid gland. A dose of 150 μc. (approximately 5 μg.) of labeled com-

TABLE 2. CLINICAL FEATURES OF PATIENTS IN WHOM THE BILIARY EXCRETION OF TRIAC AND TETRAC WAS STUDIED

Patient, age & sex	Diagnosis	Compound studied	Days postop.
E.F.—64 F	Chronic cholecystitis and cholelithiasis	Triac	18
J.C.—53 M	Chronic cholecystitis and cholelithiasis	Triac	6
L.O.—72 F	Chronic cholecystitis and cholelithiasis; staghorn calculus	Triac	3
		Tetrac	5
M.C.—87 F	Chronic cholecystitis and cholelithiasis	Triac	1
J.B.—65 M	Chronic cholecystitis and cholelithiasis	Tetrac	33

pound in 1 per cent human serum albumin was injected intravenously. Serial blood samples were drawn and total urine collections made. Aliquots of serum and urine were assayed for radioactivity in a well-type scintillation counter. A dilution of the injection solution, prepared within a few hours of use, was employed as a standard, and radioactivity in samples was expressed as a percentage of the administered dose. External body counting was performed in some patients, with a scintillation probe positioned over the lower rib margin in the right midclavicular line ("hepatic area") and over the third left intercostal space, 5 cm. from the midline ("cardiac area").

In the patients with external bile drainage, serial samples of bile and serum were assayed for radioactivity; urine collections were not made, and no external body counting was performed. These patients did not receive methimazole.

Chromatography. Selected samples of serum, urine and bile were acidified to pH 2–3 with 10*M* H_2SO_4 and extracted three times with three volumes of butanol saturated with water or with 5 per cent sodium thiosulfate. The combined extracts were taken to dryness in vacuum. The residue was dissolved in a small volume of methanol-2*N* ammonia or of pure methanol, and applied to strips of filter paper, together with appropriate stable carriers.[2] In some instances, when the residue contained much amorphous gummy debris, a larger amount of methanol was used to take up the residue, and the insoluble waste was removed by centrifugation. The supernatant was then dried to a small volume in air at room temperature or in vacuum, and applied to filter-paper strips. Samples of all discarded material were assayed for radioactivity, and none contained a significant amount.

Ascending chromatography was carried out in various solvent systems: butanol-dioxane-2*N* ammonia (4:1:5, BDA); butanol-acetic acid-water (100:12:88, BuAc); and tertiary amyl alcohol saturated with 2*N* ammonia (TAA). Most satisfactory results were obtained with BDA, but usually identification of the radioactive compounds was confirmed in one of the other two systems. Chromatographic strips were assayed for radioactivity in a strip-scanner, and radioautographs were also prepared. Positions of stable carriers were detected by spraying with 1 per cent palladium chloride, for iodide, and with diazotized sulfanilic acid followed by 10 per cent potassium carbonate, for thyronine derivatives.

TCA precipitation. Selected samples of serum were mixed with 3 volumes of 20 per cent trichloracetic acid (TCA), and the resulting precipitate was washed twice with 5 per cent TCA. The precipitate was then dissolved in 2*N* NaOH and counted in the well counter.

Beta-glucuronidase hydrolysis. Suspected glucuroconjugates were dissolved in 0.6 ml. of distilled water, to which was added 0.4 ml. of 0.4*M* phosphate buffer, pH 6.2, and 25 mg. of beta-glucuronidase[3] in 1 ml. of distilled water. In control vessels, 1 ml. of distilled water was substituted for the enzyme solution. Following overnight incubation at 37°C., aliquots were applied directly to filter-paper strips and chromatographed in the described solvent systems.

Electrophoresis of serum. Electrophoretic studies of thyroxine-binding proteins were performed on sera from several of the patients. Tracer amounts of labeled T_4, and measured increments of stable T_4, were added to portions of a given serum, and electrophoresis was performed on Whatman #3 filter paper in Tris-maleate buffer, pH 8.6 (12).

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[2] Stable Triac and Tetrac were generously supplied by Dr. Wayne A. Ruddy, Warner-Lambert Research Institute, Morris Plains, New Jersey.

[3] Obtained commercially from Sigma Chemical Co., St. Louis, Missouri.

The "binding capacity" of TBG and TBPA for thyroxine was defined as the maximal amount of stable T_4, determined from the known specific activity in each portion of serum, which could be bound by each of these proteins. Description of the apparatus used, and a more detailed discussion of the methods, will be reported elsewhere (24).

RESULTS

Testing of methods

Butanol extraction and TCA precipitation. Since it was not known whether the compounds used would be precipitated with serum proteins by TCA, or extracted quantitatively from serum by butanol, recovery of Triac and Tetrac by these methods was determined. Both Triac and Tetrac were completely extracted from serum by butanol (recoveries 100.5 and 103.0 per cent, respectively). When corrections were made for contaminating inorganic iodide, both compounds were almost completely precipitated from serum by TCA (recoveries greater than 95 per cent).

Front-running material. In early experiments, it was noted that a large amount of the radioactivity recovered from samples of serum and urine by the butanol extraction procedure migrated to the solvent front in all three of the solvent systems employed. This material was not seen in those few samples which contained sufficient radioactivity to permit direct chromatography, and thus the front-running material seemed a possible artifact of butanol extraction. To test this possibility, labeled Triac in a dilute solution of serum albumin was carried through the regular extraction procedure, and samples for chromatography were taken at each step. It was found that front-running material was formed in small amounts in the first extraction, and that very large amounts were formed as the acid extract was taken to dryness. However, neutralization of combined extracts with 2*N* NaOH prior to drying greatly diminished the formation of this front-running material. This finding agrees with the earlier suggestion that such rapidly-migrating substances may be butyl or other esters of iodophenols, formed in acid solution (25). In all subsequent studies, the combined butanol extracts were neutralized with 2*N* NaOH or NH_4OH before drying.

Triac glucuronide. In the course of the studies with Triac, it became evident that a compound resembling Triac glucuronide was an important metabolic product. The behavior of Triac glucuronide in the extraction methods employed was therefore determined. The conjugate was prepared biosynthetically in the rat. An external biliary fistula was created,[4] and the rat received an injection of 500 μc. of labeled Triac. Bile collected between two and six hours after injection was used for direct preparative chroma-

[4] The bile fistula was kindly prepared by Dr. J. D. Ostrow, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass.

tography. Neither iodide nor Triac could be definitely identified in rat bile. Rather, the major radioiodinated components were a compound migrating less rapidly than iodide and one migrating between iodide and Triac in the BDA solvent system. Both of these compounds were eluted and aliquots were subjected to beta-glucuronidase hydrolysis. The compound of lower mobility was changed by the enzyme into a substance with the mobility of Triac in BDA; the mobility of the other compound did not change detectably after incubation with the enzyme.

The presumed glucuronide of Triac was highly soluble in acid butanol (partition coefficient, butanol:water, approximately 100:1 at pH 3), was chromatographically unchanged by butanol extraction, and was 97 per cent precipitable from serum with TCA. To assess further its protein-binding characteristics, the glucuronide was eluted from filter paper into serum which was then electrophoresed in Tris-maleate buffer at pH 8.6. The protein-binding properties were qualitatively similar to those of unconjugated Triac, in that the greater part was bound to albumin, a lesser amount to TBPA, and none to TBG.

Metabolism of Triac

Labeled Triac was administered to 7 patients. Electrophoretic studies of serum from 3 of these patients showed normal binding capacities of TBG and TBPA for thyroxine. In all patients, total radioactivity in serum declined very rapidly, reaching approximately 3 per cent of the administered dose per liter in three hours (Fig. 1). The rate of disappearance of I^{131} then slowed, but remained quite rapid; the half-times (t/2), estimated from the semi-logarithmic disappearance slope, averaged 19 hours (range, 15–27 hours).

Chromatography of serum was difficult because of the very small amounts of radioactivity present after the first two to three hours. Early (3–5 minute) blood samples from 6 of the patients were chromatographed, and all showed predominantly unchanged Triac. In later samples, from one hour to six and a half hours after injection, iodide predominated, and only faint additional spots could be seen on radioautographs. In 4 patients, a compound migrating more slowly than iodide was identified. Representative findings in BDA chromatograms from one patient are shown in Figure 2. At fifty-five minutes, Triac made up only a small proportion of the radioactivity, and three other components could be seen—inorganic iodide, a compound migrating more slowly than iodide, and one migrating slightly more slowly than Triac. At six and a half hours, iodide predominated, and the only other compound present was the one with a mobility which was less than that of iodide. Such slow-moving compounds are usually thought to be conjugates. A compound in bile, of similar mobility, was demon-

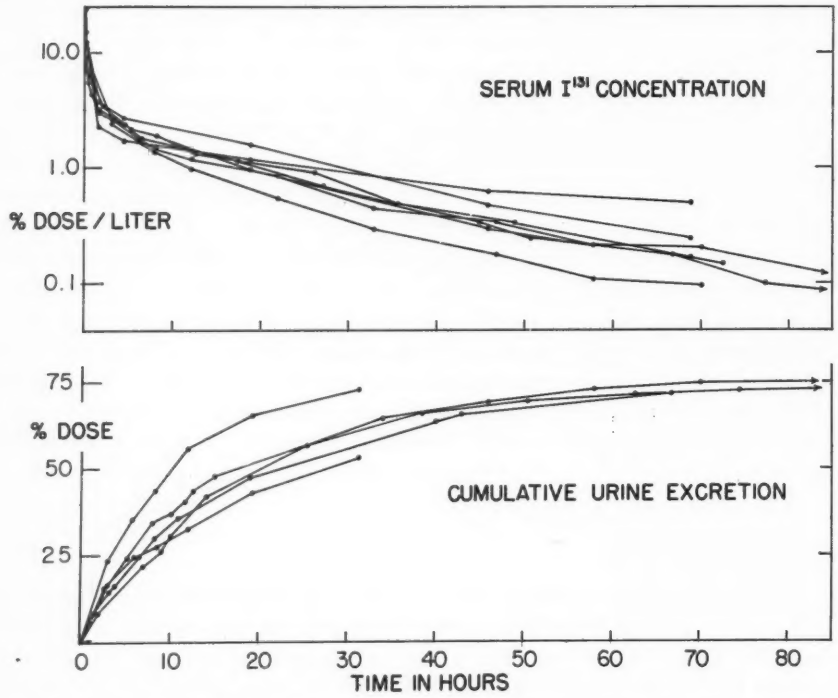

FIG. 1. Disappearance from serum and cumulative urinary excretion of radioactive iodine after single intravenous injection of tracer quantities of I^{131}-labeled Triac in normal patients. Each curve represents observations in a single subject.

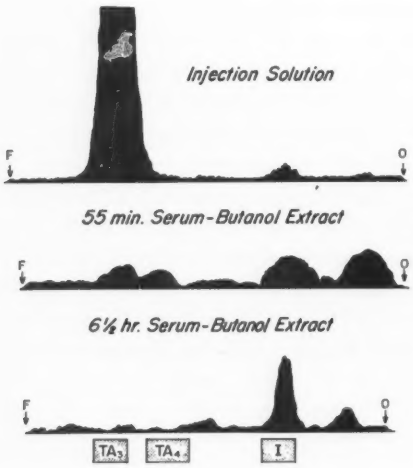

FIG. 2. Chromatography of serum following administration of I^{131}-labeled Triac. Butanol-dioxane-2*N* ammonia (4:1:5) solvent system.

strated to be a glucuroconjugate of Triac, and will be described subsequently.

The preponderance of inorganic iodide in sera obtained after the first hour was confirmed in 2 patients by TCA-precipitation. In both, TCA-precipitable radioactivity declined to 30 per cent of the total within two hours, and then rose gradually to 50 per cent of total radioactivity by sixty hours (Fig. 3). Thus, the disappearance of iodinated organic moieties from

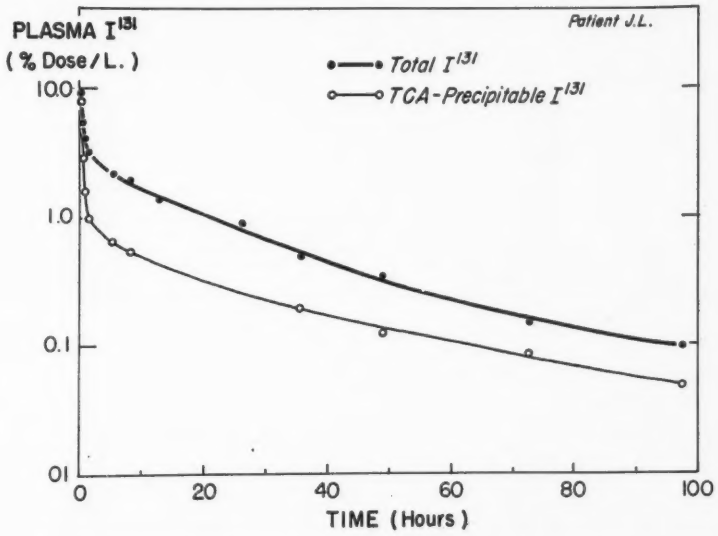

FIG. 3. Total and trichloracetic acid-precipitable I^{131} in the serum after administration of labeled Triac.

serum during the first two hours was even more rapid than measurements of total radioactivity indicated, but thereafter, total and TCA-precipitable radioactivity declined at about the same rate.

Due to the presence in serum of the multiple radioiodinated components seem on chromatograms, the true rate of disappearance and distribution space of unchanged Triac could not be determined accurately from data on either total or protein-bound radioactivity in serum. On the other hand, quantities of unchanged Triac were so low as to preclude accurate measurement, following chromatographic separation. Thus, it can only be concluded that Triac rapidly disappeared from the serum into a large distribution space.

Radioactive iodine appeared promptly in the urine after the administration of labeled Triac. Figure 1 shows the cumulative urinary excretion of

I^{131} in the 5 patients from whom adequate collections could be made. Excretory curves were almost identical, with approximately 50 per cent of administered radioactivity excreted in twenty hours, and 60 per cent within two days. The ultimate urinary excretion was estimated to be 75 per cent of the administered dose. However, precise calculation of this value, by methods previously described (4), was not possible in view of the uncertain concentration of Triac in the plasma.

Urinary I^{131} was composed mainly of inorganic iodide. A representative series of BDA chromatograms of urine from one patient (H.T.) is shown in Figure 4. The two-hour sample showed a marked predominance of iodide,

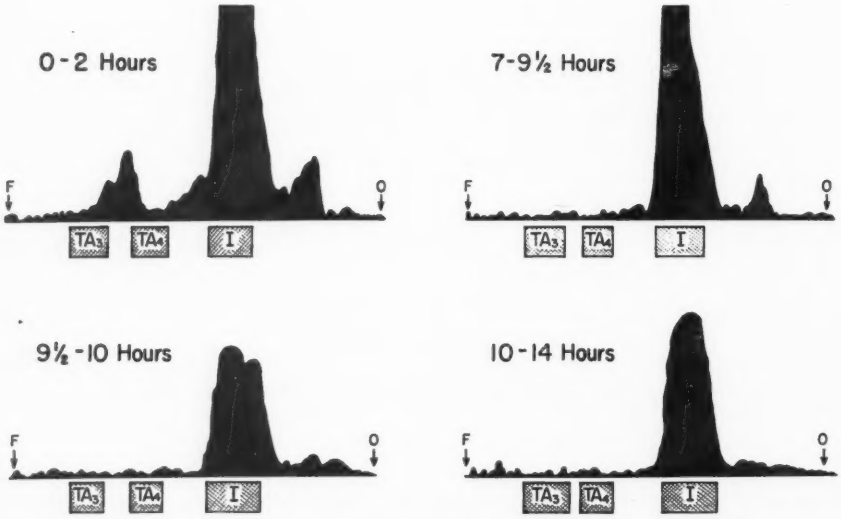

FIG. 4. Chromatography of urine following administration of I^{131}-labeled Triac. Butanol extracts chromatographed in butanol-dioxane-2*N* ammonia (4:1:5). Patient H.T.

although several other products could be seen—a substance migrating near, but probably distinct from Triac, and compounds migrating less rapidly than iodide, probably conjugates. In the three later samples, the only definite peak, other than that of inorganic iodide, was that of the slow-moving conjugate. In other patients, small amounts of unchanged Triac were seen in very early urine specimens, and several showed a distinct peak between iodide and Triac in both BDA and TAA solvent systems. The latter peak, seen also in serum (Fig. 2) and in bile (Fig. 6), corresponds in mobility to the sulfoconjugated product of Triac seen during the *in vitro* metabolism of Triac by rat kidney slices (26).

No attempt was made to measure radioactivity in the stools; however,

on the basis of the recovery from urine, about 25 per cent must ultimately have been excreted in the stools.

To determine if pharmacologic doses of Triac are metabolized in the same manner as tracer doses, a second study was performed in Patient J.L., adding 2 mg. of stable Triac to the labeled material. During the two studies, no significant differences in the rate of disappearance from serum of either total or protein-bound I^{131}, or in the rate of urinary excretion of radioiodine, could be discerned.

External body counting, performed in 4 patients in the early period after injection of Triac, revealed far more radioactivity over the hepatic area

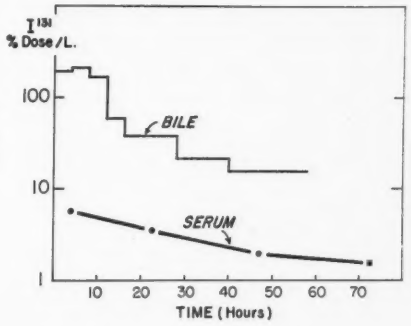

FIG. 5. Radioactivity in bile and serum following administration of I^{131}-labeled Triac. Note logarithmic scale on vertical axis. Patient J.C.

than over the cardiac area. This apparent concentration of activity in the liver led to the study of 4 patients with partial external biliary drainage. These subjects all showed a slower disappearance of radioactivity from serum than did the subjects discussed previously, possibly as a result of hepatic dysfunction. However, even in these patients, there was a remarkable concentration of radioactivity in the bile. In Figure 5 a comparison is made, on a logarithmic scale, of the concentrations of total radioactivity in successive samples of bile and serum from one patient (J.C.) in whom the concentration of radioiodine in bile collected between four and eight hours after injection reached 209 per cent of administered dose per liter, while the concentration of I^{131} in serum at four hours was only 6 per cent of the administered dose per liter. In the other 3 subjects, maximal concentrations of 115, 67 and 68 per cent of the administered dose per liter were achieved.[5]

Chromatographic studies of bile were performed only in specimens ob-

[5] More precise quantitative treatment of these data in terms such as biliary clearance, or bile:plasma ratios did not seem justified, since the fistulas were incomplete, the delay in transit time through the bile ducts and T-tube was unknown, and there were multiple radioactive components in both serum and bile.

tained during the first six to eight hours after injection. Within this time period, inorganic iodide comprised only a small proportion of biliary radioactivity. Unchanged Triac was present only during the first four hours, after which other radioiodinated components were present. Aside from Triac, the bile contained 2 compounds which migrated less rapidly than iodide, and 1 which migrated between iodide and Triac in the BDA solvent system (Fig. 6). The last of these may be a sulfoconjugate, as mentioned previously. Of the 2 remaining unknowns, the one present in greater proportion was eluted and incubated with beta-glucuronidase. As shown in Figure 6, control incubation had no effect on migration in the BDA solvent system, but incubation with the enzyme restored the chromatographic migration to that of Triac. Thus, it is likely that the eluted compound was a glucuronide of Triac.

Metabolism of Tetrac

Five euthyroid patients received labeled Tetrac. In 3 of these, electrophoretic studies of serum showed normal binding capacities of TBG and TBPA for thyroxine. The initial rate of disappearance of radioactivity from serum was much slower than that which followed administration of Triac, and after about twenty hours an apparent single exponential disappear-

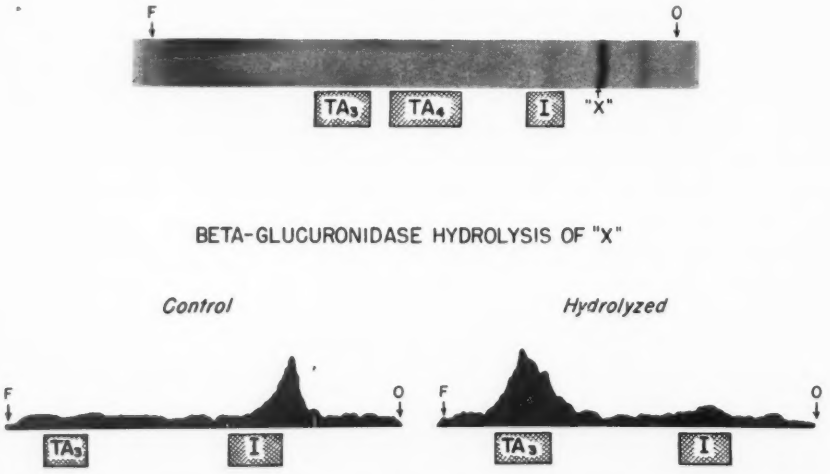

FIG. 6. Chromatography of bile after administration of I^{131}-labeled Triac; enzymatic hydrolysis of unknown compound in bile. The radioautograph of a chromatogram of a butanol extract of bile is shown at the top. Samples from Patient E.F., chromatographed in butanol-dioxane-2*N* ammonia (4:1:5). Radioactivity at solvent front is considered an artifact of butanol extraction, *cf.* text. Unknown compound "X," eluted and aliquots incubated with and without beta-glucuronidase.

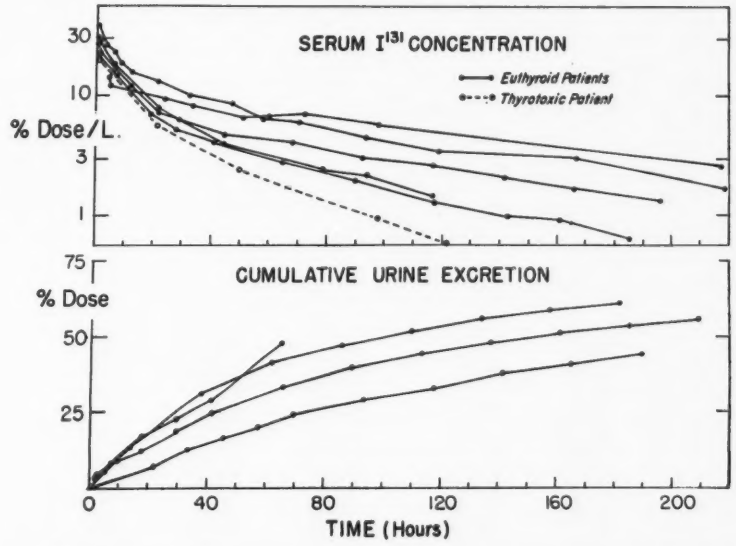

FIG. 7. Disappearance from serum and cumulative urinary excretion of radioactive iodine after single intravenous injection of tracer quantities of I^{131}-labeled Tetrac in normal patients. Each curve represents observations in a single subject. Dotted line depicts findings in a patient (R.R.) with diffuse toxic goiter, untreated.

ance rate was achieved (Fig. 7). Half-times of total radioactivity during the latter phase averaged 3.3 days (range, 2–4). Volumes of distribution, estimated by methods previously described (4), averaged 12 liters (range, 9–15).

Chromatographic analysis of radioactivity was possible in samples of serum drawn up to twenty-two hours after injection. During this period, Tetrac comprised the greater part of the radioactivity (Fig. 8). In samples of serum from one patient at twenty-two, thirty-six and sixty hours after injection, 76 to 83 per cent of the radioactivity was TCA-precipitable.

Radioactivity appeared in the urine more slowly after administration of Tetrac than after administration of Triac (Fig. 7). Calculated values for the ultimate urinary excretion averaged 67 per cent (range, 59–72 per cent) of the administered radioactivity. It is assumed that the remainder was ultimately excreted in the stool.

Chromatography of urine samples revealed that inorganic iodide was the major excretory product; Tetrac was also present (Fig. 8). In one of the patients, small amounts of material migrating less rapidly than iodide in BDA and TAA were seen in both blood and urine.

In 4 patients, external body counting was performed, and the radioactivity was approximately equal over the hepatic and cardiac areas.

One thyrotoxic patient was studied. In this patient's serum, the thyroxine-binding capacity of TBG was normal, but that of TBPA was somewhat diminished. The rate of disappearance of radioactivity from this patient's serum was more rapid than that in the euthyroid subjects, with a half-time after equilibration of 1.8 days (see dotted line, Fig. 7).

Two patients with T-tube biliary drainage were also studied. The maximal concentration of radioactivity in the bile was 21 per cent of the administered dose per liter—lower than the concentrations achieved after

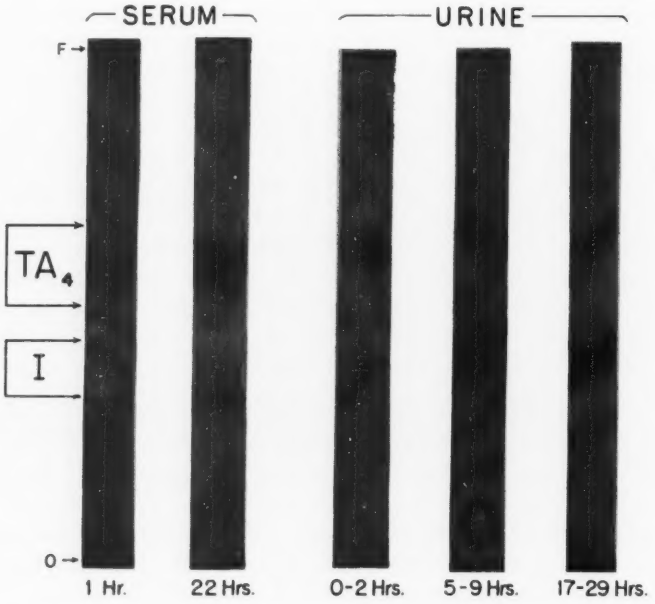

FIG. 8. Chromatography of butanol extracts of serum and urine after administration of I^{131}-labeled Tetrac. Patient J.K.

administration of Triac. In one of the two subjects (L.O.), studies were performed with both Triac and Tetrac. Triac was given first, and the concentration of radioactivity in the bile reached a maximum of 68 per cent of the administered dose per liter. However, when Tetrac was given, the maximal concentration achieved in comparable samples of bile was 20 per cent of the administered dose per liter.

Chromatography of bile samples in BDA showed Tetrac, iodide and a slowly-migrating unidentified compound. This last compound was probably a conjugate, but was not present in sufficient quantity to permit enzymatic hydrolysis.

DISCUSSION

From the present studies, several conclusions regarding the metabolism of Triac and Tetrac can be drawn. Triac disappears from the serum with great rapidity, and conjugation appears to play an important part in its metabolism. Conjugates of Triac appear in the plasma, and their presence makes difficult the quantitative assessment of the rate of disappearance and volume of distribution of unconjugated Triac. Circulating conjugates may contribute to those found in the urine, or the latter may arise from conjugation within the kidney itself. Triac is concentrated by the liver and excreted in high concentration in the bile, in both free and conjugated forms. The degree to which these compounds in bile are normally reabsorbed is uncertain, since, in the present studies, total biliary excretion was not determined. However, most of the I^{131} administered in Triac rapidly appears in the urine as inorganic iodide, implying that deiodination is the major ultimate pathway in Triac metabolism.

The rate of disappearance of TCA-precipitable I^{131} after administration of labeled Triac (t/2, less than 1 day) is more rapid than the reported rate of fall in serum PBI concentration after intravenous administration of stable Triac to athyreotic patients (t/2, 2–3 days) (16). However, myxedematous patients have a slower peripheral turnover of thyroxine than do euthyroid subjects (5, 6), and may similarly metabolize Triac more slowly.

Tetrac disappears from serum more slowly than Triac, although its rate of disappearance is more rapid than that reported for T_4 (3, 4) and is almost as rapid as that reported for T_3 (2, 3). Its volume of distribution is similar to that of T_4 (4). Conjugation is less rapidly effected during the metabolism of Tetrac than during that of Triac, and Tetrac is not so highly concentrated in bile. However, the ultimate fecal excretion of Tetrac equals or exceeds that of Triac. Like Triac, the greater part of Tetrac is ultimately deiodinated, with excretion of inorganic iodide in the urine.

Studies of this type in humans are always beset with difficulties. The limitations on the permissible amounts of radioactivity do not allow one to continue observations beyond a few days when dealing with compounds such as Triac and Tetrac, which prove to be rapidly metabolized. In addition, it is impossible in man to make direct observations on the distribution of these compounds in specific organs, or on the nature of the metabolites which may be contained therein. Consequently, the role that tissue localization plays in the unusual effects of these compounds cannot be readily assessed. However, it is evident that such studies in man do give important information that cannot be obtained from studies in laboratory animals, since there are distinct species differences both in protein-binding and in other features of hormonal metabolism. In the rat, for example, each acetic-acid derivative disappears from serum more slowly than its parent com-

pound (21), and is more slowly deiodinated (19). On the basis of the studies in rats, it could be concluded that the relative metabolic ineffectiveness of Tetrac and Triac might be related to their slower entry into cells. However, on the basis of the present studies in human subjects, an almost opposite conclusion might be drawn—that their ineffectiveness was the result of their more rapid cellular penetration and degradation. The fact may be that the peculiar effects of the derivatives are due in whole or in part to their specific interactions with cellular enzymes, rather than to characteristics of their metabolic fate.

The major stimulus to the present studies, however, was the hope that protein-binding of the deaminated derivatives, which have a peculiar affinity for TBPA, could be correlated with the characteristics of their peripheral metabolism, and that such correlations might amplify or modify current theory regarding the relation of hormonal binding to hormonal metabolism. This theory suggests that the proportion of hormone present in extracellular fluids in the free or unbound state is a major determinant of its fractional rate of uptake by cells, and ultimately of its fractional rate of disappearance from the plasma. The proportion of unbound hormone, in turn, is the resultant of the simultaneous interaction of the hormone with all of its extracellular binding proteins, and thus is determined by the total concentration of binding sites, their individual affinities for the hormone, and the concentration of the hormone itself (*cf.* reviews, references 11–13). To facilitate discussion, the net efficiency of protein-binding, as reflected in the proportion of hormone present in the bound state, will be referred to as "overall binding."

Data are available concerning the overall binding of Tetrac, Triac and their parent hormones. Christensen has compared the overall binding of these compounds by determining the rate at which labeled tracer amounts of each equilibrate across a dialysis membrane separating two aliquots of normal human serum (27). The relative proportions of free hormone, determined by this method, correlate well with another index of overall binding, the proportion of hormone taken up by red blood cells from human plasma *in vitro*. The findings by both methods are recorded in Table 3, which also summarizes data from electrophoretic studies (Tris-maleate buffer, pH 8.6) on the relative avidities of each compound for TBG and TBPA. It is apparent that failure to bind to TBG does not preclude a high degree of overall binding; among these compounds, Tetrac shows the greatest overall binding, and this probably is due chiefly to its firm bond to TBPA. The greater affinity of TBPA for Tetrac than for Triac, and the greater affinity of TBG for T_4 than for T_3, correlate well with the observation that the overall binding of the tetraiodinated compounds is greater than that of their triiodinated analogues. The finding that Tetrac disappeared from

plasma less rapidly than Triac was therefore not unexpected. However, in terms of overall binding, Tetrac is more firmly bound than T_4, and Triac more firmly than T_3. It might therefore have been expected that Tetrac would disappear less rapidly than T_4, and Triac less rapidly than T_3, but such was not the case.

Several factors might explain this unexpectedly rapid disappearance of Tetrac and Triac. First, the overall binding by serum proteins, *in vitro*, may not accurately reflect the actual proportion of free compound at cell surfaces. For example, a lower pH near actively metabolizing cells may affect binding so as to favor cellular uptake of the derivatives more than the uptake of the parent compounds. A selective effect of pH on specific binding interactions has been shown for T_4; its bond to TBPA and possibly to albumin is more sensitive to changes in pH than is its bond to TBG (13).

Second, tissue cells may bind the derivatives more avidly than they bind

TABLE 3. COMPARISONS OF *in vivo* METABOLISM AND *in vitro* PROTEIN-BINDING OF L-THYROXINE, L-3,5,3′-TRIIODOTHYRONINE AND THEIR ACETIC ACID DERIVATIVES*

Compound	Half-time in man (days)	*In vitro* binding in plasma or serum			
		Rate of dialysis (27) (%/24 hrs.)	Red blood cell uptake (27) (%)	Specific binding (15)†	
				TBG	TBPA
L-Thyroxine	6–8 (3,4)	0.50	5.3	++++	+++
L-3,5,3′-Triiodothyronine	2–3 (2,3)	6.00	(very high)‡	++	0
Tetraiodothyroacetic acid	2–4	0.05	0.6	0	+++++
3,5,3′-Triiodothyroacetic acid	<1	1.16	8.3	0	++

* Numbers in parentheses indicate reference numbers of the source from which the data cited were obtained.

† Based on electrophoretic and dialysis data presented in the reference cited. The number of plus signs indicates binding avidity, and is not precisely proportional to the intensity of the bond, but is employed to indicate the rank order of binding intensity.

‡ Absolute values not presented in reference cited.

T_4 and T_3, thus overcoming the firmer binding of the derivatives to serum proteins. The erythrocyte, although it binds the derivatives in a way that does seem to reflect the proportion of free hormone in plasma (Table 3), may not be typical of other tissues.

Third, the rates of cellular degradation and conjugation of Tetrac and Triac in human tissues may be much more rapid than those of T_4 and T_3, and may overshadow any rate-limitations imposed by protein-binding phenomena. Although dehalogenase systems from laboratory animals, including the rat, have generally been less active toward these derivatives than toward the parent hormones (28), this is accompanied, in the rat, by a

slower rate of disappearance from serum for the derivatives than for their respective parent hormones (21).

Finally, it is possible that TBPA serves as a vehicle for transporting into cells those compounds with which it is associated, much as iron-binding globulin specifically transports iron into reticulocytes (29).

These factors afford a means of accommodating the present discordant observations within the general theory that overall binding is an important determinant of the rate of hormonal metabolism. It is apparent that the same factors also complicate the interpretation of previous observations which are consistent with the theory (12). Phases of hormonal metabolism subsequent to extracellular binding are difficult to evaluate by present techniques, especially in human material. Only when information concerning these factors is complete will observations of *in vivo* metabolism provide conclusive evidence concerning the role of extracellular binding interactions in regulating hormonal metabolism.

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DIFFERENCES IN THE RESPONSE OF EUTHYROID AND HYPERTHYROID PATIENTS TO THYRO-INHIBITORY SUBSTANCES*†

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ABSTRACT

The 24-hour thyroidal uptake of radioiodine (I^{131}) was determined in control subjects and hyperthyroid patients before and after administration of either 500 mg. of thiourea or 4 Gm. of potassium thiocyanate. Thiourea abolished I^{131} uptake in 21 normal controls and in 2 patients with nontoxic goiter, whereas the mean uptake value in the hyperthyroid group (8 patients) was above 20 per cent. Potassium thiocyanate reduced the uptake values below 10 per cent in 18 euthyroid controls and 7 patients with nontoxic goiter, whereas in 15 hyperthyroid patients the values were significantly higher. The inhibitory effect of thiocyanate appeared to vary directly with the serum concentration of the ion in both the hyperthyroid and control groups. This difference in response to thiocyanate or thiourea may be a useful means to distinguish between normal subjects and patients with hyperthyroidism.

PREVIOUS studies in man have shown that the thyrotropin-stimulated thyroid gland may incorporate radioiodine after the administration of inhibitory amounts of thiocyanate (1) or thiourea (2). In view of these findings, it seemed worth while to study the effect of thyro-inhibitory substances upon thyroid function in thyroid disease. The effect of standardized amounts of thiourea and potassium thiocyanate upon the uptake of radioiodine (I^{131}) by the thyroid gland was determined in normal subjects and in hyperthyroid patients. The results of these studies indicated that thyroidal I^{131} uptake in the normal subjects was almost completely abolished, whereas in the hyperthyroid subjects the glands continued to take up appreciable amounts of radioiodine.

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MATERIALS AND METHODS

Potassium thiocyanate

Following 24-hour baseline I^{131} uptake determinations, 18 normal controls, 15 patients with unequivocal hyperthyroidism, and 7 patients with nontoxic goiter received a single 3-Gm. oral dose of enteric-coated potassium thiocyanate.[1] Twenty-four hours later a second dose (1 Gm.) was given along with a tracer dose of carrier-free radioiodine[2] (I^{131}) and the thyroidal I^{131} uptake was measured on the following day. The technique for measuring both the baseline and the repeated I^{131} uptakes has been outlined in a previous publication (1). The concentration of thiocyanate in the serum was determined by means of a standardized spectrophotometric method (3).

Thiourea

The thiourea was made up in gelatin capsules and given in a single 500-mg. oral dose to 21 euthyroid controls, 8 patients with previously untreated thyrotoxicosis and 2 patients with nontoxic goiter. The tracer I^{131} was administered three to four hours following the thiourea, and the uptake was measured twenty-four hours from the time that the thiourea had been ingested. On several occasions a dose of 300 mg. of potassium perchlorate was given at the time of the uptake determination in order to confirm the fact that the radioiodine within the thyroid gland was organically bound.

RESULTS

Potassium thiocyanate

The response of the hyperthyroid group to potassium thiocyanate was distinctly different from that of the control groups (Fig. 1). Both the euthyroid subjects and patients with nontoxic goiter exhibited mean thyroidal radioiodine (RAI) uptake values which were below 10 per cent, whereas the mean value for the hyperthyroid group was above 20 per cent. There were no discernible differences between the values for some of the subjects in the control group and those for the hyperthyroid group after thiocyanate. In order to determine whether or not the overlapping of values might be related to variations in serum thiocyanate concentrations, blood was collected from some of the subjects twenty-four hours after the administration of potassium thiocyanate. Analysis of the sera revealed that there was a marked variability in serum thiocyanate concentration among the euthyroid and the hyperthyroid patients (Fig. 2). When the thyroidal I^{131} uptake values were plotted against the serum thiocyanate concentrations (Fig. 3), it became apparent that in each group the inhibitory effect of thiocyanate varied directly with the level of the ion in the serum.

Thiourea

The results shown in Figure 4 indicate that a single 500-mg. dose of thiourea virtually abolished the 24-hour I^{131} uptake in the normal controls

[1] Potassium Thiocyanate Enseals (0.2-Gm. tablets), Eli Lilly and Co.. Indianapolis.
[2] Abbott Laboratories, Oak Ridge, Tenn.

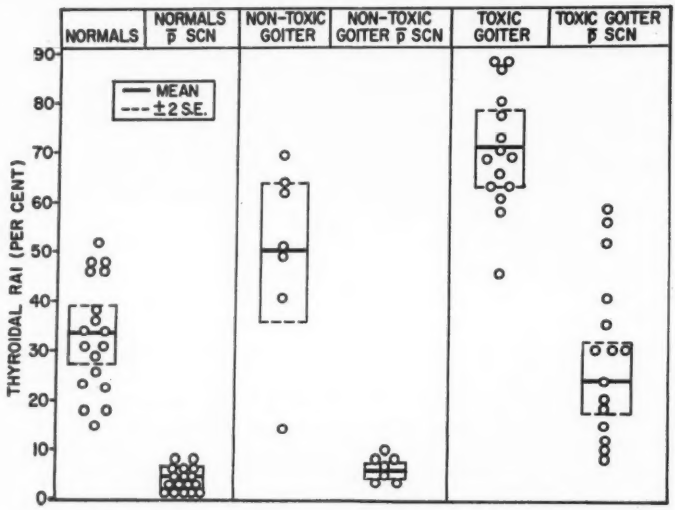

FIG. 1. *Response to potassium thiocyanate.* The 24-hour thyroidal radioiodine (RAI) uptake values before and after 4 Gm. of potassium thiocyanate are shown for each group of subjects. Each circle represents the 24-hour RAI value for a single subject. The baseline RAI values are listed in the left half of each column under the respective headings.

and in the patients with nontoxic goiter (not shown), whereas the mean uptake value in the hyperthyroid group was above 20 per cent.

DISCUSSION

Earlier studies in normal subjects had shown that the 24-hour uptake of radioiodine by the thyroid gland was almost completely inhibited at serum thiocyanate concentrations above 5 mg. per 100 ml. (1) and that a 500-mg.

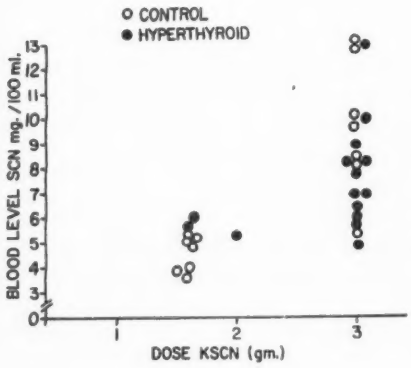

FIG. 2. *Serum levels of thiocyanate.* Each circle represents the thiocyanate concentration (vertical axis) in serum collected 24 hours after the administration of a dose of potassium thiocyanate (horizontal axis) to control subjects. The solid black dots represent values for hyperthyroid patients.

oral dose of thiourea abolished normal thyroid function (2, 4). The study described here indicates that the hyperthyroid gland was able to incorporate significant amounts of radioiodine in spite of the administration of potassium thiocyanate or thiourea. Although the thyro-inhibitory materials succeeded in impairing the function of the hyperthyroid gland, they were unable to abolish it completely. A similar pattern of thyroidal behav-

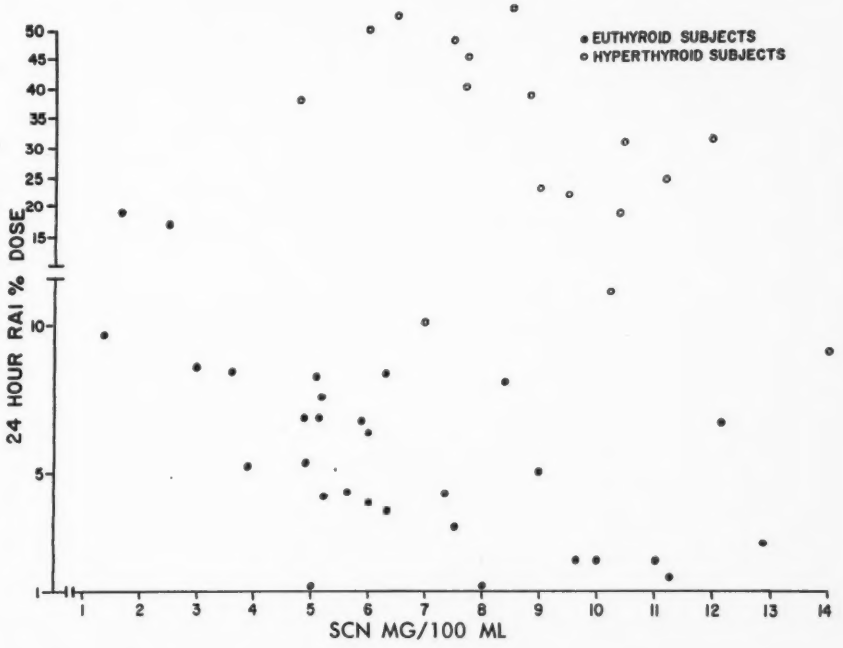

FIG. 3. *Effect of thiocyanate upon the 24-hour thyroidal radioiodine uptake.* Each solid black dot represents the RAI uptake (vertical axis) in a control subject with a particular serum level of thiocyanate (horizontal axis). Each circle represents the RAI uptake in a hyperthyroid patient.

ior had been noted previously in the normal subjects treated with thyrotropin before receiving thiourea or thiocyanate. Neither the latter observations nor those reported here can be fully explained by the available data. However, in guinea pigs receiving thyrotropin, both thiocyanate-S^{35} and thiourea-S^{35} were degraded more rapidly than they were in control animals (5, 6). These observations suggest that the hyperfunctioning thyroid gland may metabolize the thyro-inhibitory materials at an accelerated rate. Hence, in the case of the hyperthyroid patient or in the thyrotropin-treated normal subject, an augmented rate of degradation of thiourea and thio-

cyanate might conceivably reduce the effective intracellular concentration of these materials, thereby permitting some degree of thyroid function. Other factors may also affect the response of the hyperthyroid gland to thiourea or thiocyanate, *i.e.*, the rapid turnover of iodine in the hyperfunctioning thyroid gland may compensate for the reduction in function induced by the inhibitory materials.

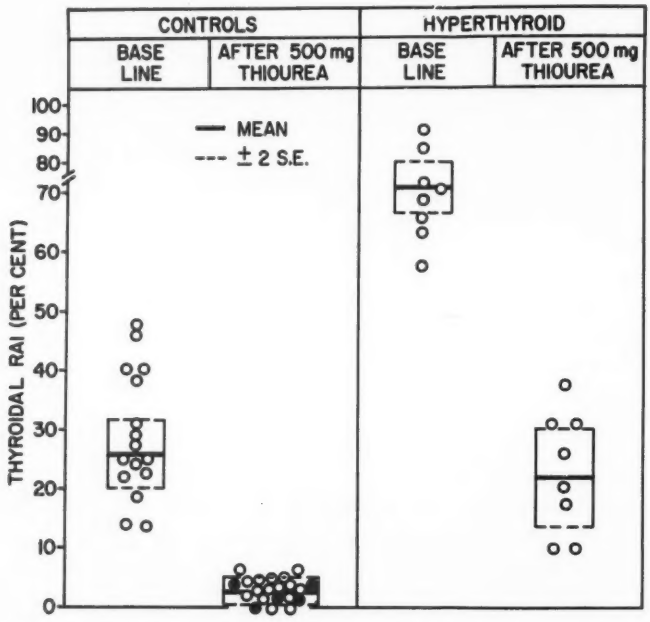

FIG. 4. *Response to thiourea.* The 24-hour thyroidal RAI uptake values before and after a single 500-mg. dose of thiourea are shown for the control and hyperthyroid groups. Each circle represents the value for a single subject. The solid black dots shown at the bottom right-hand half of the "Controls" column designate the subjects in whom baseline RAI uptake determinations were not made.

It is of interest that some of the differences in I^{131} uptake values between the euthyroid and the hyperthyroid groups following administration of thiocyanate were not as distinct as they were with thiourea. In view of the variations in serum thiocyanate concentration following the 4-Gm. dose of potassium thiocyanate, it seems reasonable to believe that overlapping of the thyroidal I^{131} uptake values in some instances might be attributed to an inability to achieve or maintain uniform serum levels of the ion. The reasons for the inconsistent serum thiocyanate levels are not clear, but several possibilities may be considered, among which are: 1) differences

in the rate and quantity of potassium thiocyanate absorbed from the gastrointestinal tract; 2) differences in the rates of metabolism of thiocyanate ion; 3) differences in the volume of distribution of thiocyanate ion; and 4) differences in the renal clearance of the thiocyanate ion. Apparently the thiocyanate ion disappears more slowly from the serum of hypothyroid patients and more rapidly from the serum of hyperthyroid patients than it does from the serum of normal subjects (unpublished data).

It is noteworthy that some of the patients were less affected by thiourea than were others. This observation may have some clinical significance with regard to treatment with antithyroid drugs. It is conceivable that the uptake of radioiodine by the thyroid gland in some hyperthyroid patients receiving antithyroid compounds may be related to the differences in the rates of intrathyroidal degradation of these substances (7); presumably, the more quickly the antithyroid substances are metabolized, the shorter their duration of action within the thyroid gland.

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COMPONENTS OF THE SERUM PROTEIN-BOUND IODINE FOLLOWING ADMINISTRATION OF I^{131}-LABELED HOG THYROGLOBULIN*

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ABSTRACT

Following the oral administration of I^{131}-labeled hog thyroglobulin to 3 healthy human subjects, the labeled products found in the blood were only in part thyroxine. The other labeled substances were not identified, but they were neither iodoaminoacids nor their degradation products. The clinical implication of these findings is discussed.

ALTHOUGH thyroxine is the normal product of the thyroid gland found in the blood (1–7) it is not established that thyroxine is the major component of the serum protein-bound iodine (PBI) during oral administration of desiccated thyroid. Albert and associates (8) fed labeled thyroglobulin to humans in order to observe the rate of absorption and the disposition of the labeling I^{131}, but qualitative observations on the nature of the products in blood following administration of thyroglobulin have not been reported. This study was undertaken to ascertain whether organic iodinated substances other than thyroxine may contribute to the serum PBI under these circumstances. Chromatography and electrophoresis demonstrated that thyroxine is only one of several substances comprising the PBI following oral administration of I^{131}-labeled hog thyroglobulin.

CLINICAL MATERIAL AND METHODS

Subjects

Two adult women and 1 man were studied; all were under 35 years of age. They were in good health, and their serum PBI values were normal.

The subjects were given 5 drops of saturated solution of potassium iodide daily for three days prior to each study in order to block recirculation of iodide. They were then given the I^{131}-labeled test substance (hog thyroglobulin or thyroxine) in physiologic sodium chloride solution. Immediately following the administration of the labeled substance, each subject was given 15 mg. of desiccated thyroid in a tablet. The desiccated thyroid was given simply as a carrier substance in order to assure that each subject received a significant amount of thyroxine. At the time that the labeled thyroglobulin

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was prepared, the iodine content was not known. Both iodine and protein content were measured after the patient had received the determined amount of radioactivity. Twenty-four hours following the administration of labeled material, approximately 50 ml. of blood was withdrawn. Serum was prepared and frozen prior to study.

Two studies were carried out on 2 subjects, and only 1 study was performed on the third. All 3 subjects received I^{131}-labeled thyroglobulin first. Six weeks later, 2 subjects received I^{131}-labeled thyroxine by mouth.

Methods

Labeled thyroxine. Exchange labeled thyroxine was purchased from Abbott Laboratories, Oak Ridge, Tennessee. The 50 per cent propylene-glycol solution in which it was supplied, containing 20 μc. of I^{131}, was diluted to 50 ml. in physiologic sodium chloride solution; this contained only a trace of thyroxine. The subject drank the entire 50 ml. No carrier thyroxine was added.

Labeled hog thyroglobulin. A 25-pound pig was given 5 mc. of sodium iodide^{131} subcutaneously four days prior to killing it by a blow on the head. The thyroid was removed and I^{131}-labeled thyroglobulin was prepared by fractionation of the soluble protein in ammonium sulfate (9). Following the final precipitation at 42 per cent saturation with ammonium sulfate, the protein was dissolved in 24 ml. of physiologic saline and centrifuged at 105,000 g for three hours at 4° C. More than 90 per cent of the radioactivity was recovered in the sediment by this procedure. The sedimented protein was then dissolved in 50 ml. of physiologic saline. It migrated electrophoretically as a homogeneous protein with the characteristics of thyroglobulin. The concentrations of protein (10), iodine (11) and radioactivity were determined; the latter was measured by comparing the counting rate in an aliquot of the prepared 50-ml. solution with the counting rate in a mock iodine gamma standard equivalent to .04 μc. of I^{131}. The amount of solution containing 50 μc. of I^{131} was determined and was administered as previously noted. The specific activity of the I^{131}-labeled hog thyroglobulin was 6.7 mc. I^{131}/mg. I^{127}/335 mg. protein. The total iodine given in labeled thyroglobulin was 7.5 μg. The total protein-incorporated iodine given was approximately 37 μg.

Resin chromatography and protein precipitable iodine. Methods of resin chromatography and trichloracetic acid (TCA) precipitation of labeled substances in serum have been previously described (7).

Electrophoresis of serum proteins. On wide glass plates measuring 16×12 inches, a piece of Whatman #3 Mm paper was soaked in veronal buffer at pH 8.6, ionic strength 0.10. Along a 10-inch line 3 inches from the center of the paper, 0.5 ml. of serum was distributed. Following equilibration, the circuit was closed at 120 volts for fifteen to eighteen hours at room temperature. The papers were dried and inspected under ultraviolet light for straightness of the albumin front. If this was straight, strips were cut from the upper and lower margins of the paper and stained with bromphenol blue. The remaining large, center portion of the sheet was then cut into 24 half-inch strips from the cathodal margin of the paper to the anodal end. These strips were folded and counted in a well-type scintillation counter at the .360 MEV peak of I^{131}. Since all strips were of the same size, the counts per minute (less background) in each strip were plotted simply as a function of the strip without further interpolation. The stained upper and lower strips were also marked in half-inch segments from the cathodal to the anodal margin of the paper. The amount of radioactivity localized to corresponding stained protein spots on the papers was calculated. The protein areas localized by staining were gamma globulin, $alpha_1$ and $alpha_2$ globulin, albumin, and the areas between these spots.

TABLE 1. COMPARISON OF I^{131}-LABELED IODINE COMPOUNDS IN SERUM THAT WERE PROTEIN PRECIPITABLE AND RECOVERABLE BY RESIN CHROMATOGRAPHY FOLLOWING ORAL ADMINISTRATION OF LABELED THYROXINE

In both instances the amount of serum used for chromatography was 5 ml. Therefore the number of counts recovered by chromatography was five times greater than indicated. (T_3+T_4) I^{131} represents I^{131} localized by resin chromatography to fractions in which tri- and tetra-iodothyronines are characteristically eluted.

Subject	TCA* I^{131} cpm/ml. serum	(T_3+T_4) I^{131} cpm/ml. serum
P. Bo.	274	257
A. Gr.	222	211

* Precipitable with trichloroacetic acid.

RESULTS

Thyroxine

Following the oral administration of labeled thyroxine the only iodinated organic substances which appeared in the blood, as identified by chromatography, were labeled thyroxine and 3:5:3 -triiodothyronine. The recovery of TCA-precipitable iodine as I^{131}-labeled iodothyronines was quantitative in the 2 subjects studied. The data are presented in Table 1 and Figure 1. When these sera were subjected to electrophoresis, the labeled substance was almost entirely localized in the inter-alpha globulin (Fig. 2).

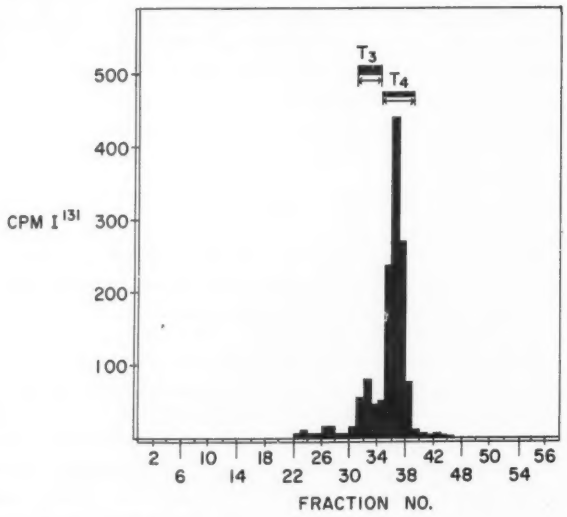

FIG. 1. I^{131} recovered as iodothyronines during resin chromatography of serum following oral administration of labeled thyroxine.

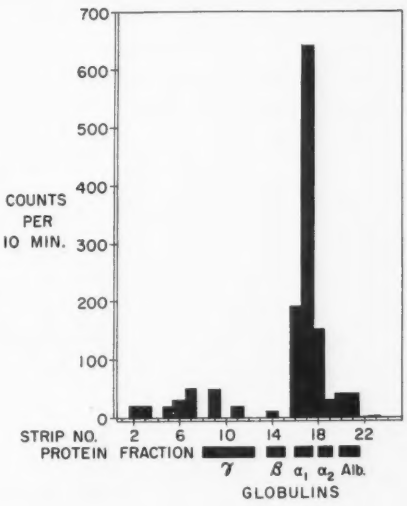

FIG. 2. Amount of I^{131} recovered in various paper strips of a serum electrophoretic analysis following the administration of labeled thyroxine.

The areas of the paper in which various protein fractions were localized by staining are indicated by labeled bars below the main graph.

Hog thyroglobulin

Following the oral administration of labeled hog thyroglobulin, labeled thyroxine was only one of several constituents which appeared in the blood. The proportion of TCA-precipitable iodine recoverable as iodothyronines varied from 39 to 70 per cent, as shown in Table 2. Protein precipitable substances other than iodothyronines were therefore also present in the blood. Such substances were not recoverable by resin chromatography.

In Figure 3 are shown schematic representations of the electrophoreses of sera following administration of hog thyroglobulin. The inter-alpha globulin, which normally contains virtually all of the thyroxine (12), contains only a fraction of the total label. This is not due to overloading of the serum with thyroxine and spill-over into the other proteins, since labeled thyroxine when fed with the same amount of desiccated thyroid was

TABLE 2. COMPARISON OF I^{131}-LABELED IODINE COMPOUNDS IN SERUM THAT WERE PROTEIN PRECIPITABLE AND RECOVERABLE BY RESIN CHROMATOGRAPHY FOLLOWING ORAL ADMINISTRATION OF LABELED THYROGLOBULIN

(See legend to Table 1 for corresponding details.)

Subject	TCA* I^{131} (cpm/ml.)	(T_3+T_4) I^{131}		TCA $I^{131}-(T_3+T_4)^{131}I=PBI^{131}$ which is not iodothyronine	
		cpm/ml.	%	cpm/ml.	%
P. Bo.	185	130	70	55	30
A. Gr.	253	136	54	117	46
H. Jo.	201	78	39	123	61

* Precipitable with trichloroacetic acid.

localized in the inter-alpha globulin (Fig. 2). The additional amount of thyroxine given in the labeled thyroglobulin was less than 3 μg.[1]

The largest amount of labeled material localized in one spot, other than that localized in the inter-alpha area, was found in an area ahead of the

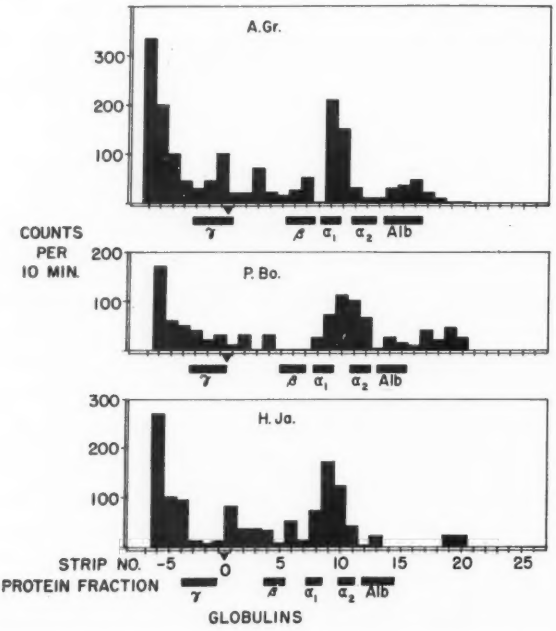

FIG. 3. Graphs of the type shown in Figure 2, except that serum was obtained following the oral administration of thyroglobulin.

gamma-globulin toward the cathode. In 2 of the subjects (A. Gr. and H. Jo.) this was apparently the major iodinated substance detected in the serum by electrophoresis. In the sera of all 3 subjects there was a small amount of labeled material about the origin, in the albumin fraction and ahead of the albumin fraction toward the anode.

DISCUSSION

The two types of organic iodinated compound found in the blood of normal subjects twenty-four hours following the administration of a single dose of I^{131}-labeled thyroglobulin were:

1. Thyroxine isolated in characteristic fractions during chromatography

[1] The amount of thyroxine in the desiccated thyroid was estimated to be 30 per cent of the organic iodine measured.

and in the inter-alpha globulin during electrophoresis.

2. Unidentified iodinated substances which migrate with serum proteins other than the inter-alpha globulin during electrophoresis and which are not recoverable in any portion of the eluate of the chromatography system used.

Thyroxine itself does not give rise to any non-thyroxine products in the blood, other than a small amount of 3:5:3′-triiodothyronine. The non-thyroxine products found following the administration of labeled hog thyroglobulin are not iodotyrosines, iodothyronines or iodohistidines, since these may be differentiated by the chromatographic technique used. These unusual substances are not derived from thyroxine; they are not simple iodoaminoacids; yet they probably are hydrolytic products of thyroglobulin.

Since diiodotyrosine is the major iodinated constituent of thyroglobulin (13), there is a possibility that such unusual blood products may be degradation products of the iodotyrosines. When approximately 0.5 mg. of labeled diiodotyrosine is fed to a normal subject, the only labeled substance recovered in the blood twenty-four hours later is inorganic iodide.[2] This is in accord with the observation that parenterally administered diiodotyrosine is rapidly deiodinated (14). The non-thyroxine iodinated substances observed here are not derived from diiodotyrosine. Because iodohistidines are present in the thyroid gland in only trace amounts (15), it seems unlikely that they are derived from this aminoacid.

There is no further information to indicate whether these substances may be simple proteins, peptides or substances of another chemical nature.

Varying amounts of thyroxine and other iodinated substances were present in the blood of the 3 subjects, and any particular numerical value for the serum PBI level might reflect different effective circulating hormonal levels. The variations in the quality of the PBI and in possible metabolic effect of each constituent make it unlikely that the PBI in persons producing endogenous thyroid hormone can be compared to the PBI of persons taking desiccated thyroid orally. Endogenously produced thyroid hormone is almost entirely thyroxine (7). The PBI in these subjects who received thyroglobulin by mouth was at most only 70 per cent and at least 40 per cent thyroxine. Although the serum PBI level in normal and in hyperthyroid subjects can be equated with the circulating level of thyroxine, the same correlation can obviously not be made in subjects taking desiccated thyroid.

There is no apparent reason why these results following the administration of one dose of thyroglobulin may not be applied to patients who take

[2] Because the results of this study (in a fourth patient) were negative, the details of the experiment are not described.

desiccated thyroid daily. There is no information available indicating how long these unidentified compounds may persist in the blood. However, since persons who take desiccated thyroid do so daily, any specific blood sample would be drawn within twenty-four hours of a preceding dose of thyroid. It seems likely, therefore, that similar qualitative results would be obtained in such patients. The possibility is being investigated that prolonged daily administration of desiccated thyroid may yield different qualitative results.

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THE EFFECTS OF VARIOUS CHLORINE DERIVATIVES OF THYRONINE ON SERUM CHOLESTEROL CONCENTRATION AND MYOCARDIAL STIMULATION IN RATS AND HUMANS

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ABSTRACT

Chlorinated and iodinated thyronine derivatives were studied for their effects on serum and liver cholesterol and on hypertrophy of the cardiac ventricle in the rat. Compounds characterized by higher ratios of cholesterol to cardiac effects were found among the derivatives with the D-configuration and with chlorine atoms in the 3 and 5 positions. In the rat, some compounds—notably 3:5-dichloro-3′:5′-diiodo-D-thyronine (DCl_2I_2T) and 3:5:3′-trichloro-5′-iodo-D-thyronine (DCl_3IT)—exerted a much greater effect on cholesterol metabolism than on the myocardium. DCl_2I_2T, 3:5:3′-trichloro-D-thyronine (DCl_3T) and 3:5-dichloro-D-thyronine (DCl_2T) were administered to 30 hypercholesterolemic men with coronary heart disease. Although DCl_2I_2T produced no significant reduction of serum cholesterol concentration, DCl_3T and DCl_2T did; and all 3 substances caused symptoms of myocardial stimulation, usually angina. Therefore none of these compounds is regarded as suitable for administration to patients with coronary heart disease. DCl_3T and DCl_2T produced striking depression of the serum protein-bound iodine level, and this effect is discussed.

THE metabolic effects of many iodinated analogs of thyroxine have been studied in the rat, in hypothyroid patients and in euthyroid men with coronary heart disease. In contrast, only little attention has been given to other halogenated derivatives of thyroxine. Previous work (1–3) has shown that certain dextro-isomers, such as 3:5-diiodo-D-thyronine and D-thyroxine, reduce serum and liver cholesterol levels and yet produce less stimulation of the myocardium than do many levo-isomers. The results of laboratory tests carried out in an attempt to discover drugs with relatively greater effects on serum and liver cholesterol levels than on the myocar-

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dium are presented here, together with the results of clinical investigations in which serum cholesterol levels and thyroactivity were studied in man.

STUDIES IN ANIMALS

Methods and Material

Methods

The methods and animals used in this work were, except when otherwise indicated, similar to those previously described (3). Oxygen consumption rates were determined on a number of animals by the method of Tomich and Woollett (4). Test compounds were all administered by admixture with the diet, and various activities of the test compounds investigated were assessed by observations of the mean responses noted in groups of 10 animals at each of a number of graded dose levels and compared with those in similar groups of animals receiving different doses of a "standard" compound. Except when otherwise indicated, comparisons were made with 3:5-diiodo-D-thyronine (DT_2) as the standard compound, and the diet used was diet B (arachis), *i.e.*, a semi-synthetic diet containing added cholesterol, cholic acid, choline and arachis oil (5).

Compounds tested

(All compounds were administered by mixing with the diet either the compound or a solution of it in ethanol and 0.1*N* NaOH, 1/1, diluted with water.)

3:5-Dichloro-L-thyronine	LCl_2T
3:5-Dichloro-D-thyronine	DCl_2T
3:5:3′-Trichloro-L-thyronine	LCl_3T
3:5:3′-Trichloro-D-thyronine	DCl_3T
3:5:3′:5′-Tetrachloro-L-thyronine	LCl_4T
3:5:3′:5′-Tetrachloro-D-thyronine	DCl_4T
3-Chloro-5-iodo-L-thyronine	LClIT
3-Chloro-5-iodo-D-thyronine	DClIT
3-Chloro-5:3′:5′-triiodo-L-thyronine	LClI_3T
3-Chloro-5:3′:5′-triiodo-D-thyronine	DClI_3T
3:5:3′-Trichloro-5′-iodo-D-thyronine	DCl_3IT
3:5-Dichloro-3′-iodo-D-thyronine	DCl_2IT
3:5-Dichloro-3′:5′-diiodo-D-thyronine	DCl_2I_2T
3:5-Diiodo-3′-chloro-D-thyronine	DI_2ClT
3:5-Diiodo-3′:5′-dichloro-D-thyronine	DI_2Cl_2T
3:5-Diiodo-D-thyronine	DT_2
D-Thyroxine	DT_4

The chlorinated thyronines were prepared by the methods described by Dibbo and associates (6).

Results

Chlorothyronines

In this series of experiments the test compound and the standard compound (DT_2) were administered in the diet at not less than three different dose levels to groups of 10 rats each. These rats were then used for evaluation of serum and liver cholesterol levels and cardiac ventricle weight after a fourteen-day test period. The activities of the test compounds (in terms

of the standard) in depressing serum and liver cholesterol concentration or in elevating cardiac ventricle weights, and the ratios between the cholesterol depressant and ventricular hypertrophy effects, are shown in Table 1. Each result denotes the mean values from tests in which the mean activity in depressing serum cholesterol concentration was determined from the doses of test and standard compound needed to induce levels of 200 mg. and 300 mg. of cholesterol per 100 ml. of serum. Liver cholesterol depressant effects were assessed from the doses needed to induce levels of 3 Gm. and 4 Gm. of cholesterol per 100 Gm. of liver. Ventricular hypertrophy was assessed from the doses needed to induce ventricle weights of 450 mg. and 500 mg. per 100 Gm. of body weight.

Iodo-chlorothyronines

Tests were also carried out on a number of D-thyronine derivatives containing both chlorine and iodine, because DCl_2T and DCl_3T showed higher ratios of anti-cholesterol to cardiac effects than had previously been noted in any of the iodinated compounds studied, and it was hoped that the introduction of iodine into the molecule might increase activity without unfavorably affecting the ratio between the two effects. These investigations (Table 1) showed that replacement of chlorine by iodine in the D-thyronines increased activity, and that in most instances this increase in activity was

TABLE 1. ACTIVITY OF COMPOUNDS IN PREVENTING A RISE OF SERUM AND LIVER CHOLESTEROL LEVELS AND IN CAUSING AN INCREASE OF CARDIAC VENTRICLE WEIGHT IN FEMALE RATS GIVEN DIET B (ARACHIS) FOR 14 DAYS, AND RATIOS FOR CHOLESTEROL LEVEL VERSUS VENTRICLE WEIGHT

Compound	No. of tests	Activities (mg. of DT_2 having same effect as 1 mg. of test compound)			Ratios	
		Serum cholesterol (SC)	Liver cholesterol (LC)	Ventricle wt. (V)	SC/V	LC/V
LCl_2T	1	1.0	—	2.5	0.4	—
LCl_3T	1	0.7	—	4.5	0.15	—
LCl_4T	1	0.15	—	0.22	0.7	—
DCl_2T	7	1.2	0.5	<0.4	>3	>1.2
DCl_3T	5	2.7	2.9	1.0	2.7	2.9
DCl_4T	2	0.12	0.2	<0.3	>0.4	>0.7
DCl_2IT	3	6.5	9.4	20	0.32	0.47
DCl_2I_2T	4	4.0	8.0	1.5	2.7	5.3
DCl_3IT	2	0.9	2.3	0.25	3.6	9.2
DT_2	—	1.0	1.0	1.0	1.0	1.0
DI_2ClT	2	1.9	3.9	11	0.17	0.35
DI_2Cl_2T	2	0.60	0.7	<0.7	>1.0	>0.8
DT_4	11	4.0	7.7	4.3	0.93	1.8
DT_3	10	78	53	56	0.68	0.72
LClIT	1	0.5	0.95	3.2	0.16	0.3
DClIT	2	0.8	0.75	0.65	1.2	1.2
LClI_3T	1	5.0	1.8	16	0.31	0.11
DClI_3T	1	4.9	1.6	3	1.60	0.53

more pronounced with respect to ventricular hypertrophy than cholesterol levels. Further, the compounds containing 3 halogen atoms were more active than those containing 4, and the increased action was particularly marked on the heart, so that the mixed trihalogen compounds usually had less favorable activity ratios. Of the compounds tested, DCl_2T, DCl_3T, DCl_2I_2T, DCl_3IT and $DClI_3T$ had the highest ratios of anti-cholesterol to heart effects, and of these DCl_2T, DCl_3T and DCl_2I_2T were selected for clinical trials because they had the most favorable ratios combined with either relatively high potency or ease of preparation or both.

Effects of DCl_3T *and* DT_4 *in thyroidectomized rats*

Forty weanling female albino rats were used for this experiment. Thirty-five of these animals were surgically thyroparathyroidectomized and 5 were subjected to only a sham operation. Seven days later, all the thyroidectomized animals were each treated with 50 μc. of I^{131}. Immediately after operation, and thereafter, the thyroidectomized animals received 0.2 per cent calcium gluconate in the drinking water, the sham-operation animals being given tap water. After thyroidectomy or sham operation all the rats were maintained with the stock diet for fourteen days. At the end of this period the sham-operation rats were given diet B (arachis), and the thyroidectomized animals were randomly allocated to 7 groups, each of 5 animals; these were then randomly allotted to one or other of the 7 treatment groups, which were all given diet B (arachis) with or without additions of either DT_4 or DCl_3T, as indicated in Table 2. The mean oxygen consumption rates of all groups were determined by the method of Tomich and Woollett (4) immediately before starting the various treatments and again after eight days of the test. All animals received treatment for twenty-seven days, to allow time for development of sudanophilic lesions in the cardiovascular system. They were then killed for investigation of serum and liver lipids, measurement of organ weights, and assessment of the sudan score to measure the extent of the sudanophilic lesions. The results of these observations are summarized in Table 2. The hearts were preserved in formalin, so that cardiac ventricle weights here refer to weights of the ventricle after fixation in formalin, whereas all other organs were weighed fresh. Some hearts were reserved for histologic examination, and the ventricle weights of these could not be recorded. The results of this experiment (Table 2) show that both DT_4 and DCl_3T increased the growth rate of thyroidectomized animals and, dependent upon the doses used, increased the oxygen consumption rate to levels similar to or greater than those of the control animals. At the same time, the weights (per 100 Gm. of body weight) of the kidney, liver, spleen and cardiac ventricle were increased, and the weight of the pituitary was depressed, as would be ex-

TABLE 2. EFFECT OF dT_4 AND dCl_3T ON MEAN SERUM AND LIVER CHOLESTEROL LEVELS, LIVER TOTAL LIPID, SUDAN SCORE, AND ORGAN WEIGHTS OF THYROIDECTOMIZED RATS MAINTAINED FOR 27 DAYS WITH DIET B (ARACHIS), AND EFFECT ON OXYGEN CONSUMPTION RATE AFTER 8 DAYS OF TREATMENT

(each group initially contained 5 animals)

Group	Addition per Kg. of diet	Body wt. (Gm.)		Organ wt. per 100 Gm. of body wt.						Serum cholesterol (mg./100 ml.)	Liver cholesterol (Gm./100 Gm.)	Liver total lipid (Gm./100 Gm.)	Sudan score	Oxygen consumption (L/rat/hr.)	
		Initial	Final	Kidney (Gm.)	Liver (Gm.)	Spleen (Gm.)	Adrenals (mg.)	Pituitary (mg.)	Cardiac ventricle* (mg.)					day 0	day 8
1**	Nil	102	151	1.04	5.43	0.69	26	6.7	296†	281	3.73	20.8	2.5	1.75	2.14
2	Nil [1]‡	86	95	0.92	4.85	0.40	23	11.6	284†	697	4.88	21.2	6.5	1.00	1.54
3	dT_4, 6.25 mg.	91	152	0.95	5.33	0.74	28	8.3	297†	295	3.60	19.8	1.6	1.05	2.44
4	dT_4, 25 mg. [1]	108	175	1.00	5.89	0.70	29	7.6	293	228	3.77	19.3	0.5	1.11	2.54
5	dT_4, 100 mg. [2]	84	166	1.25	7.16	0.92	31	7.5	374	246	3.42	17.0	0.8	0.98	2.72
6	dCl_3T, 12.5 mg. [1]	75	120	1.17	5.67	0.73	27	9.9	284	358	3.88	19.5	2.6	1.05	1.67
7	dCl_3T, 50 mg.	77	145	0.99	5.72	0.77	34	9.3	258	255	3.58	17.4	2.6	0.92	1.95
8	dCl_3T, 200 mg. [2]	91	162	1.15	6.36	0.97	19	7.3	330	190	3.19	16.4	0.5	1.03	2.54

* Ventricles weighed after fixation in formalin.
** This group consisted of rats subjected to a sham operation.
† One heart from each group not weighed.
‡ Numbers in brackets denote the number of deaths occurring during the test.

pected from previous observations on the effects of the natural thyroid hormones (7, 8).

Comment

The values in Table 1 show that LCl_2T, LCl_3T and LCl_4T were more active in promoting ventricular hypertrophy than the corresponding D compounds, but members of the D series were not less active than those of the L series in depressing serum cholesterol levels. Thus the ratios of anti-cholesterol to cardiac effects were greater for the D compounds than for the L series. Furthermore, it may be seen that for the D series of chlorinated thyronines the effects on liver cholesterol closely paralleled those on serum cholesterol. A curious feature of these observations was the increased activity, in all respects studied, of both DCl_2T and LCl_2T over DCl_4T and LCl_4T respectively, whereas in the iodinated series, both LT_4 and DT_4 were more active than LT_2 and DT_2 (3). Both LCl_3T and DCl_3T were more active than the di- and tetra-chlorinated compounds in their effects on the heart, and in this way resembled the iodinated compounds LT_3 and DT_3 in relation to the corresponding di- and tetra-iodinated compounds. DCl_3T also had more effect on the serum cholesterol level than had either DCl_2T or DCl_4T, but LCl_3T in these tests was not more active than LCl_2T in this respect; however, this finding was based on only one experiment, so that further observations are required to determine whether this compound is significantly less active than LCl_2T. The results of the cardiac ventricle assays in this series of tests on DCl_3T, LCl_3T, DCl_4T and LCl_4T are in fair agreement with those obtained for DLCl_3T and DLCl_4T by Mussett and Pitt-Rivers (9) in the goiter-prevention assay. The replacement of a chlorine atom by iodine in DCl_2T and LCl_2T led to an increased effect on the heart, as shown by the results with D and LClIT, though this change did not greatly modify the depressant action on serum cholesterol concentration. Both D and LClIT also resembled DCl_2T and LCl_2T in that the L compound had the greater effect on the heart and the D compound the greater effect on cholesterol levels. This relation was also evident between D and LClI_3T; here the L compound was about five times as active as the D compound on the heart, whereas there was little difference in their effects on cholesterol levels. All the chlorinated thyronines and the iodo-chloro derivatives studied in the present work resembled each other in that the ratio of anti-cholesterol effect to heart effect was greater for the D than for the L series, as shown for the D and L iodinated thyronines compared in previous work (1, 3).

The comparisons obtained between compounds in these tests could not be subjected to standard statistical analysis because of the curvature and non-parallelism of the dose-response relationships noted with the different

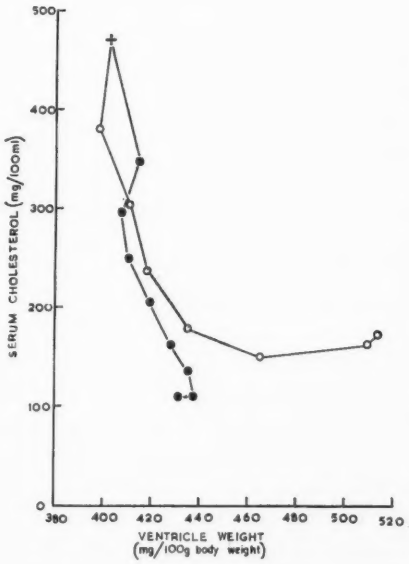

FIG. 1. Combined comparisons of DT_2 (circles) with DCl_2T (black dots) from 7 experiments. The plus sign represents values for the cholesterol-cholate-arachis oil diet alone.

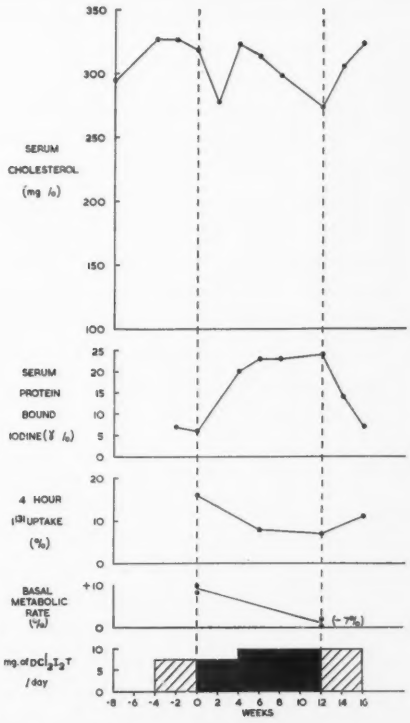

FIG. 2. Some effects of the administration of 3:5-dichloro-3′:5′-diiodo-D-thyronine to 6 hypercholesterolemic euthyroid men with coronary heart disease. Angina developed in 2 patients and atrial fibrillation in another, when receiving 10 mg. daily. Hatched areas represent placebo treatment.

compounds. As in the previous study (3), therefore, the mean serum cholesterol levels in the various groups of animals given the test and standard compound, DT_2, were plotted against the mean heart weights noted in the same groups of animals. Figure 1 summarizes the results of all available comparisons between DT_2 and DCl_2T plotted in this way. The data show that there was no significant difference at low response levels between the relations of heart weight and serum cholesterol, in that both compounds had a pronounced effect on the serum cholesterol level with only a slight effect on the heart. At higher response levels, DT_2 had a greater effect on the heart for a given depression of the serum cholesterol level than did

DCl_2T. Similar relations were noted for DCl_3T and DCl_2I_2T, thus confirming the better separation of effects noted with these compounds compared with DT_2.

It is difficult to make generalizations as to the relation between the positions of the halogen atoms and activity, but replacement of iodine by chlorine in the 3:5 positions did not necessarily lead to a fall in activity as measured by effects on cholesterol, though this change was followed by a decrease of activity measured by the cardiac ventricle test. For example, DCl_2I_2T and DCl_2T had as much effect on the serum cholesterol level as had DT_4 and DT_2 respectively, though with much less effect on the heart.

The action of the compounds in the fourteen-day tests summarized in Table 1 could conceivably be due, not to a direct thyroactive effect, but to some other mechanism such as release, activation or increased synthesis of thyroactive hormone. Although such explanations appeared unlikely, the tests summarized in Table 2 were carried out to determine whether the effects of one of the compounds, DCl_3T, could be demonstrated in thyroidectomized rats, and the opportunity was taken to compare the activity of this compound with that of DT_4 in the same test.

Both DT_4 and DCl_3T were effective in depressing serum and liver cholesterol levels, total lipid levels and the sudan score for thyroidectomized rats. Thus they displayed thyroactivity qualitatively similar to that noted with L-thyroxine and 3:5:3′-triiodo-L-thyronine in other studies (3, 10). The number of animals used in this test was not sufficient for more precise comparison of the effects of the two compounds, but the results agree with the relative potencies found as a result of the shorter tests with euthyroid animals (Table 1). From these observations it may be concluded that DT_4 and DCl_3T are thyroactive in the same sense as the natural hormones, and that the effects consequent on their administration are not mediated by way of the thyroid gland or preformed thyroid hormones.

STUDIES IN HUMAN SUBJECTS

Methods and Material

Three thyroactive substances, DCl_2I_2T, DCl_3T and DCl_2T, were administered to 30 euthyroid men who had electrocardiographic evidence of old myocardial infarction or ischemia after exercise. None of these men sustained a myocardial infarct within the six months before the administration of these substances, and all were engaged in regular employment, although some had effort angina. They were selected from the coronary disease population on account of hypercholesterolemia [serum levels graded at 270 per 100 ml., see Oliver (11)]. Before the administration of a chlorothyronine, 3 or more determinations of serum cholesterol were obtained over a ten-week period.

The men were admitted to the hospital for two nights so that the basal metabolic rate (BMR) could be measured on two consecutive mornings at rest in bed; this was done on each of the two days immediately before, and each of the two penultimate days

during the administration of the compound under study. A 12-lead electrocardiogram was recorded before, during the middle, and at the end of every study. When these euthyroid men (all out-patients) attended the hospital every two weeks, they were seen by the same physician (M.F.O.), and on each occasion particular attention was paid to subjective complaints, such as angina, exertional breathlessness, tremor and anxiety. Unknown to themselves, but known to the physician, all patients received inert tablets or capsules identical in size, shape and color to the active preparation, at the beginning and end of each study.

Serum cholesterol concentration was estimated by the Sperry and Webb (12) modification of the Sperry-Schoenheimer procedure, and serum protein-bound iodine by the chloric-acid digestion method of Zak and Boyle (13) and Grossmann and Grossmann's (14) brucine modification of the Chaney (15) ceric-sulphate technique. The thyroidal uptake of radioactive iodine was obtained from the neck-thigh ratio four hours after the oral administration of 20 μc. of I^{131}. The BMR was determined with a standard Benedict-Roth apparatus by the linear formula of Aub and Du Bois (16).

Results

*3:5-Dichloro-3':5'-diiodo-*D*-thyronine* (DCl_2I_2T)

This analog was administered for twelve weeks to 6 euthyroid men with coronary heart disease (Fig. 2). An oral daily dose of 7.5 mg. produced a significant fall in the serum cholesterol level, but this was not sustained. It recurred, however, when the daily dose was increased to 10 mg. There was a striking rise in the level of serum protein-bound iodine, and at the same time there was a slight fall in the four-hour thyroidal I^{131} uptake. The BMR decreased from an initial level of +9 per cent to +2 per cent. Effort angina developed in 2 of the 6 men, and in another subject atrial fibrillation developed when he was receiving 10 mg. daily. The increased incidence of angina subsided a few days after substitution of inert tablets for the active compound. After administration of digitalis and withdrawal of DCl_2I_2T, normal sinus rhythm returned in the patient who had atrial fibrillation.

*3:5:3'-Trichloro-*D*-thyronine* (DCl_3T)

This chlorothyronine was administered to 6 euthyroid men for sixteen weeks (Fig. 3). An oral daily dose of 20 mg. produced a significant fall in the serum cholesterol level, but this was not maintained despite an increase in dosage to 30 mg. daily. There was a gradual and significant decrease in the concentration of serum protein-bound iodine, but no significant alteration was observed in the four-hour thyroidal I^{131} uptake. The BMR decreased by 6 per cent. None of the hypercholesterolemic men with coronary heart disease complained of decreased effort tolerance, and in none did effort angina develop.

DCl_3T was then administered for twenty-two weeks to 6 more euthyroid men with coronary heart disease (Fig. 4). The oral daily dose ranged from

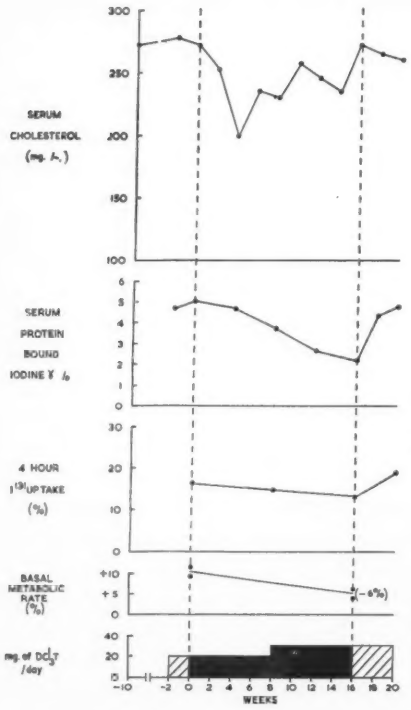

FIG. 3. Some effects of the administration of a small dose of 3:5:3′-trichloro-D-thyronine to 6 hypercholesterolemic euthyroid men with coronary heart disease. None complained of decreased effort tolerance. Hatched areas represent placebo treatment.

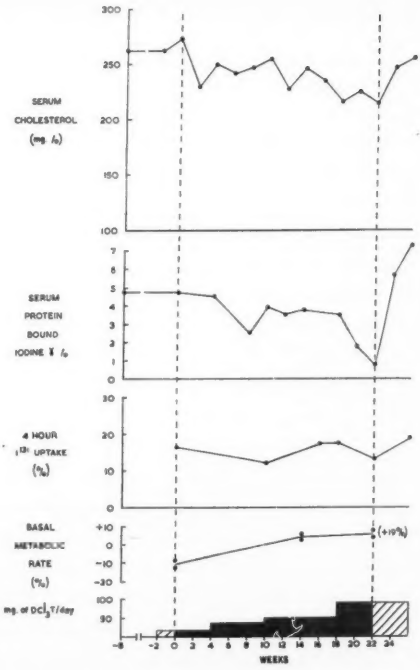

FIG. 4. Some effects of the administration of a large dose of 3:5:3′-trichloro-D-thyronine to 6 hypercholesterolemic euthyroid men with coronary heart disease. Angina developed in one, and reduced effort tolerance in another, when receiving 80 mg. daily. Hatched areas represent placebo treatment.

20 to 80 mg. The serum cholesterol level gradually fell and by the twenty-second week was 20 per cent lower than before treatment. The concentration of serum protein-bound iodine gradually fell from an initial level of 4.8 μg. per 100 ml. to 0.8 μg.; subsequently, after withdrawal of DCl_3T, it rose steeply to a mean level of 7.3 μg. per 100 ml. No significant change occurred in the four-hour thyroidal I^{131} uptake. The BMR increased by 19 per cent. While the 80-mg. dosage of DCL_3T was being administered, one man experienced a striking increase in effort angina, and another complained of unusual exertional breathlessness; both these complaints lessened when the patients received inert pills.

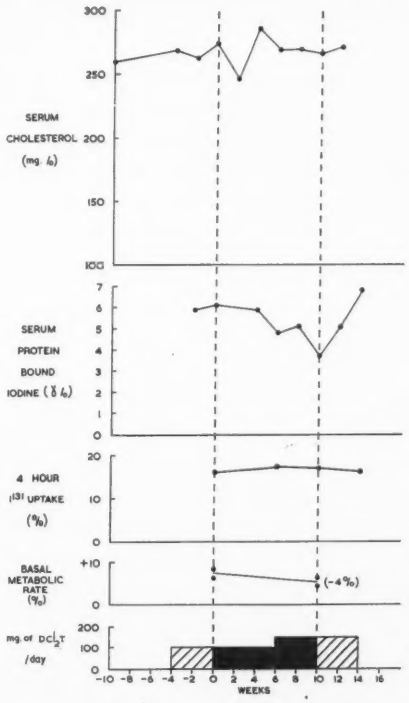

FIG. 5. Some effects of the administration of a small dose of 3:5-dichloro-D-thyronine to 6 hypercholesterolemic euthyroid men with coronary heart disease. None complained of decreased effort tolerance. Hatched areas represent placebo treatment.

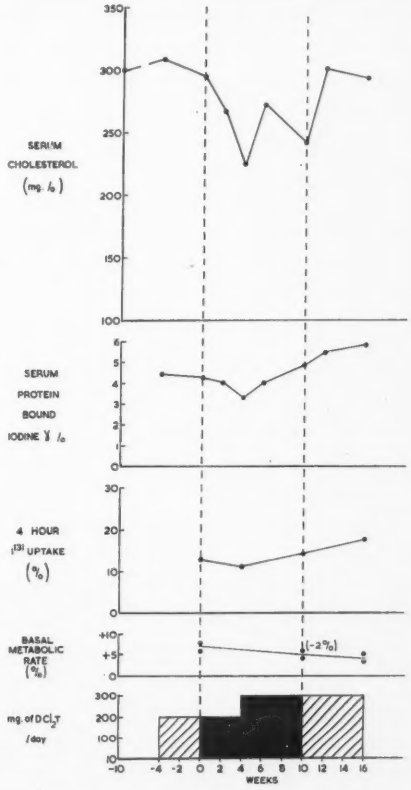

FIG. 6. Some effects of the administration of a large dose of 3:5-dichloro-D-thyronine to 6 hypercholesterolemic euthyroid men with coronary heart disease. Effort angina developed in 1 patient when he was receiving 300 mg. daily. Hatched areas represent placebo treatment.

3:5-Dichloro-D-thyronine (DCl_2T)

This chlorothyronine was administered to 6 euthyroid men for ten weeks (Fig. 5). The oral daily dose ranged from 100 to 150 mg. Although there was a slight initial fall in the level of serum cholesterol, this was not sustained and was not regarded as significant. The concentration of serum protein-bound iodine fell from an initial level of 6.1 μg. per 100 ml. to 3.7 μg., and then rose abruptly after withdrawal of DCl_2T. No significant change occurred in the four-hour thyroidal I^{131} uptake. The BMR fell by 4

per cent. None of the 6 men complained of any decrease in exercise tolerance, and in none did effort angina develop.

DCl_2T was then administered for ten weeks to 6 more euthyroid men with coronary heart disease (Fig. 6). The oral daily dose was 200 or 300 mg. The serum cholesterol level fell by 17 per cent and, after withdrawal of DCl_2T, rose to the pretreatment value. Although there was a slight initial fall in the serum protein-bound iodine level, this was not maintained. There was no significant change in the four-hour thyroidal I^{131} uptake. The BMR fell by 2 per cent. More severe and more frequent effort angina developed in 1 patient.

DISCUSSION

The administration of DCl_2I_2T to hypercholesterolemic men with coronary heart disease did not produce any significant or consistent fall in serum cholesterol concentration and was associated with features of myocardial stimulation in 3 out of the 6 patients studied. The administration of DCl_3T and DCl_2T to hypercholesterolemic men with coronary heart disease produced a significant depression in the serum cholesterol level but also led to myocardial stimulation and symptoms of angina or breathlessness. Despite the promising results of the animal assays, it is concluded that none of these 3 thyroactive compounds is suitable for reduction of the serum cholesterol level in men with coronary heart disease.

Though the rise in serum protein-bound iodine concentration during the administration of DCl_2I_2T can be explained by the comparatively large amount of iodothyronine administered, the fall in protein-bound iodine concentration during the administration of DCl_3T and DCl_2T is harder to explain. It is possible that this fall was due to replacement of protein-bound iodine compounds by the relatively large doses of chlorinated compounds administered, or their chlorinated metabolic products. An alternative explanation is that at these dosage levels the chlorinated compounds behaved like iodinated thyronines and acted as a stimulus for reducing the production of thyroid-stimulating hormone (TSH), which was reflected by a decrease in the level of serum protein-bound iodine. Although the thyroidal I^{131} uptake was not significantly affected, it may be that the validity of this test in the presence of large amounts of chlorinated thyronines could be questioned. If these chlorinated thyronines do in fact modify the responses of the pituitary-thyroid axis, it is possible that large doses of these compounds might have some therapeutic value in cases of suspected overproduction of TSH, such as exophthalmic ophthalmoplegia.

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THYROXINE AND TRIIODOTHYRONINE TURNOVER STUDIES IN DYSTROPHIA MYOTONICA*†

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ABSTRACT

The turnover of thyroxine and triiodothyronine, the extrathyroidal thyroxine space, the thyroidal uptake of radioiodine, the radioiodine conversion ratio, and the serum protein-bound iodine level were normal in 4 patients with dystrophia myotonica, despite a low basal metabolic rate. The patients were relatively unresponsive to the administration of desiccated thyroid or triiodothyronine.

OF THE several forms of muscular dystrophy, dystrophia myotonica alone is accompanied regularly by overt manifestations of disordered endocrine function. Weakness and wasting of muscle, myotonia, cataracts and coronal baldness are characteristic of this disease, but in addition testicular atrophy with increased gonadotropin excretion, diminished 17-ketosteroid excretion and decreased basal metabolism with occasional frank myxedema may be present (1–7). In contrast to the depressed basal metabolic rate, the thyroidal radioiodine uptake and the serum protein-bound iodine level are in the euthyroid range. In order to learn more about the metabolic disorder in dystrophia myotonica, turnover studies and extrathyroidal pools were determined in 4 patients with this disease after administration of I^{131}-labeled thyroxine or triiodothyronine.

MATERIALS AND METHODS

The subjects of this study were 4 adult males afflicted with a slowly progressive and unremitting myopathy affecting the distal musculature of the extremities and the temporal, masseter and sternocleidomastoid muscles most severely, in association with

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myotonia principally in the hands, calves and jaw. The myotonia was substantiated by the electromyographic findings. Three of the 4 patients (except S.G.) had relatives with the disease. All had testicular atrophy. Coronal baldness and posterior lenticular opacities were present in 3 of the 4 patients (except D.K.). All 4 complained of constipation. With the exception of Patient A.L. who also suffered from narcolepsy, tiredness and sleepiness were not problems. Dry skin, facial puffiness, enlargement of the tongue, pain in the joints, and intolerance of cold were not present. Pertinent laboratory studies are summarized in Table 1.

Turnover studies were performed as outlined by Sterling and Chodos (8), using commercial radiothyroxine and radiotriiodothyronine (Abbott Laboratories, Oak Ridge,

TABLE 1. PERTINENT LABORATORY DATA ON 4 PATIENTS WITH DYSTROPHIA MYOTONICA

	Patient D.K.	Patient A.L.	Patient S.G.	Patient B.C.
Sex & Age	M,31	M,38	M,45	M,42
Weight (lbs.)	142	151	159	153
Duration of disease (yrs.)	18	25	16	17
Electromyogram	Myotonia	Myotonia	Myotonia	Myotonia
Basal metabolic rate (%)	−19	−22	−24	−29
24-Hr. I^{131} uptake (% dose)	27.8	52.2	18.0	51.1
24-Hr. conversion ratio (%)	40.7	20.2	17.5	24.5
Serum protein-bound iodine (μg./100 ml.)	4.6	5.0	5.6	4.3
Urinary 24-hr. creatinine excretion (Gm.)	1.04	1.12	0.92	1.10
Urinary 24-hr. creatine excretion (mg.)	89	58	151	114
Serum aldolase (U/ml.)	2.7	2.9	1.7	
Serum lactic dehydrogenase (U/ml.)	117	90	102	
Serum transaminase (U/ml.)	10	14	16	
Serum cholesterol (mg./100 ml.)			160	250
Circulating thyroid antibodies	None	None	None	None
Chromatin pattern	Male	Male	Male	Male
Electrocardiogram	Normal	Normal	Normal	Normal

Tenn.). These materials were found to be essentially radiopure upon paper chromatography (9). A dose of approximately 5 μc. of I^{131} labeled *l*-thyroxine or *l*-triiodothyronine was given for each test. After administration of labeled thyroxine, 24-hour urine collections and blood samples were obtained for twelve days; after administration of labeled triiodothyronine, 12-hour urine specimens and blood samples were obtained for five days. No drugs were taken by the subjects for at least three months preceding the study.

After the completion of the turnover studies, the patients were treated with 25 μg. of triiodothyronine four times daily for six weeks, and re-evaluated. Then they were treated with 60 mg. of desiccated thyroid three times daily for eight weeks, and again re-evaluated.

RESULTS AND DISCUSSION

Although all 4 patients had moderately low basal metabolic rates ranging from −19 to −29 per cent, the thyroidal radioiodine uptake and conversion ratio at twenty-four hours, and the serum protein-bound iodine level were not abnormal (Table 1). The turnover of radiothyroxine ranged from a half-time of 6.07 to 9.36 days with a mean of 7.42 days, representing turnover rates ranging from 7.41 to 11.42 per cent per day with a mean of 9.57 per cent per day (Table 2). The extrathyroidal thyroxine distribution space ranged from 7,102 ml. to 10,690 ml. with a mean of 8,568 ml., and the

extrathyroidal pool of thyroxine ranged from 328 to 466 μg. with a mean of 414 μg. These results are in the lower range for normal subjects as reported by Sterling and Chodos (8) and are comparable to the findings in patients with extrathyroidal hypometabolism (10, 11). The thyroxine turnover and pool size in the dystrophic patients were *not* in the hypothyroid range. The turnover of triiodothyronine was somewhat more rapid than that observed by Sterling *et al.* (12) in normal subjects. No attempt was made to determine the triiodothyronine space, but it is conceivable that this was contracted. No thyroid antibodies were found in the sera of our patients.[1]

The serum aldolase, lactic dehydrogenase and transaminase levels, which are often elevated in other forms of muscular dystrophy (13), were not

TABLE 2. THYROXINE AND TRIIODOTHYRONINE TURNOVER STUDIES IN 4 PATIENTS WITH DYSTROPHIA MYOTONICA

Patient	Turnover half-time (days)		Turnover rate (% per day)		Extrathyroidal thyroxine pool	
	T_3	T_4	T_3	T_4	(ml.)	(μg.)
D.K.	1.14	7.05	60.8	9.84	7146	328
A.L.	1.37	6.07	50.6	11.42	9334	466
S.G.		9.36		7.41	7102	398
B.C.		7.21		9.62	10690	460
Mean		7.42		9.57	8568	414

abnormal in these patients. It is of interest that the sex chromatin pattern[2] in our patients was male, corresponding to the phenotypic sex as previously reported in patients with dystrophia myotonica (14).

Upon treatment with desiccated thyroid or triiodothyronine, an increase in appetite and a lessening of constipation occurred in all patients. The patient with narcolepsy (A.L.) became less drowsy. However, there was no improvement in muscle strength or myotonia. The basal metabolic rate was virtually unchanged in 2 or the 3 patients studied, and in the third the increase in BMR was less than is usually seen after such doses of thyroid medication (Table 3). The general unresponsiveness of patients with dystrophia myotonica to exogenous thyroid has been observed previously (3, 6, 7).

Thyroxine turnover and pool measurements may be added to the tests of thyroid function which yield normal results in dystrophia myotonica. In view of all these normal values for thyroid function, the low metabolic rate in this disease has been attributed to extrathyroidal hypometabolism (6, 7), even though this syndrome is poorly characterized (15) and there is as yet no direct evidence of unresponsiveness of peripheral tissues to

[1] We are indebted to Dr. Frank Schmid for determination of thyroid antibodies.

[2] We are indebted to Dr. Albert Gerbie for determination of the sex chromatin pattern.

TABLE 3. BASAL METABOLIC RATES BEFORE AND AFTER THYROID REPLACEMENT THERAPY

	Patient S.G.	Patient D.K.	Patient A.L.
Control BMR (%)	−24	−19	−22
BMR after 6 wks. of triiodothyronine, 25 μg. four times daily	−28	−12	+7
BMR after 8 wks. of desicc. thyroid, 60 mg. three times daily	−18	−22	−3

thyroxine. The lack of responsiveness to exogenous thyroid substance supports such an interpretation. It is possible, however, that diminished muscle mass (16) may be the cause of the low basal metabolic rate.

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CARCINOMA OF THE THYROID GLAND: A STATISTICAL STUDY OF 390 PATIENTS*

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ABSTRACT

At the University of California Hospitals a study has been made of the 390 patients with thyroid carcinoma who were observed in the 40-year period between 1920 and 1959. Thyroid carcinoma was found in both lobes at the time of the "initial operation" in 19 per cent of these patients, and regional lymph node metastases were reported at one time or another in 42 per cent. These data warrant a more radical surgical approach to thyroid carcinoma. During the past fifteen years, therefore, the majority of such patients at the University of California have been treated by more radical forms of thyroidectomy. The rate of local recurrence in the thyroid area was significantly lower in patients treated by total thyroidectomy than in those treated only by unilateral subtotal lobectomy. Cumulative survival rates were higher for patients with papillary carcinoma than for those with follicular carcinoma, and the survival rates for patients with papillary or follicular carcinoma were higher than for those with anaplastic carcinoma. Thyroid carcinoma is a lethal disease despite prolonged survival of many of the patients. Eventually 19 per cent of the 390 patients died of thyroid carcinoma (papillary 11 per cent, follicular 33 per cent and anaplastic 76 per cent).

CLINICAL and pathologic studies on patients with thyroid carcinoma reported from the University of California Hospitals have been designed chiefly to delineate the natural history of the 3 major forms of the disease. It was recognized that evaluation of various forms of therapy, mainly surgical, was not possible in a limited number of patients, even when they were observed for long periods after treatment.

The present report is based upon a continuing study at the University of California Hospitals of 390 patients with thyroid carcinoma who were observed in the 40-year period between January 1920 and December 1959. This study was concerned mainly with certain features of the natural history of the disease, including detailed survival data. An attempt was made to determine whether or not the frequency of neoplastic recurrence in the thyroid gland has been affected by the more radical surgical procedures employed during the past ten to fifteen years.

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MATERIALS AND METHODS

The 390 patients with thyroid carcinoma were first seen at the University of California Hospitals between January 1920 and December 1959. The hospital records and follow-up information received through June 1960 provided the clinical data. The majority of patients had been observed by members of the Thyroid Clinic.

All available histologic sections were reviewed. Thyroid carcinoma was classified into 3 main histologic types (papillary, follicular, or anaplastic), generally following the classification proposed by Warren and Meissner (1) with modifications previously made in this laboratory (2). In addition, follicular carcinoma was further subdivided into 3 groups: a) localized follicular carcinoma (invasive adenoma); b) invasive follicular carcinoma; and (c) medullary (solid) carcinoma, as defined by Hazard and coworkers (3).

The onset of carcinoma was assumed to coincide with the onset of goiter in most patients with papillary and follicular carcinoma. Multiple adenomas or chronic thyroiditis, however, accounted for longstanding goiter in some cases. In these instances, the onset of carcinoma was estimated as the time that recent thyroid growth began. For patients with anaplastic carcinoma, the onset of comparatively recent thyroid growth was considered to be the time of onset of carcinoma.

"Initial operation" was defined as including the initial surgical procedure (including biopsy of the thyroid gland or lymph node) and all subsequent surgical procedures performed within two months following the initial procedure and within two months of each other. Thus multiple procedures classified as an "initial operation" were performed within a six-month period.

Classification of the stages of thyroid carcinoma was based upon clinical, operative and pathologic findings available at the time of the "initial operation." The stages were defined as:

1. *Localized*—neoplasm confined entirely to thyroid gland.
2. *Regional*—neoplasm extending beyond the capsule of the thyroid gland: *a*) into regional lymph nodes; *b*) into surrounding organs or tissues; or *c*) a combination of *a* and *b*, without further spread.
3. *Distant*—neoplasm that had spread to parts of the body remote from the primary site.

"Early" regional and distant metastases were those noted at the "initial operation" or within two months postoperatively. Metastases noted after this period were designated as "late."

Tabulations of clinical and pathologic data were facilitated by use of IBM punch cards.

Cumulative survival rates were calculated by the actuarial life table method described by Berkson and Gage (4). Standard errors of the survival rates were computed by the method of Merrell and Schulman (5). Comparisons of survival rates (2) and chi square tests used in other comparisons were performed at the 5 per cent level of significance. Comparisons of survival rates to determine significant differences were made only for 10, 20, 30 and 40 years after the onset of carcinoma and after the "initial operation."

RESULTS

Histologic type of carcinoma and sex of patient

Of the 390 patients in this study, 286 (74 per cent) had papillary carcinoma, whereas only 5 per cent (21 of 390) had anaplastic carcinoma

(Table 1). Of the 83 follicular carcinomas, 18 were of the medullary type (3). Female patients with papillary and follicular carcinomas outnumbered males in approximately a 3:1 ratio. There were no male patients in the anaplastic group.

Histologic type of carcinoma and age of patient

Distributions according to the age of the patient at onset and age at the time of the "initial operation" are shown in Tables 2 and 3. Papillary

TABLE 1. THYROID CARCINOMA: HISTOLOGIC TYPE IN RELATION TO SEX OF PATIENT

Histologic type	Total No. of patients	Males	Females
All types (total)	390	89	301
Papillary	286	69	217
Follicular, total	83	20	63
Localized	39	9	30
Invasive	26	6	20
Medullary	18	5	13
Anaplastic	21	—	21

TABLE 2. THYROID CARCINOMA: AGE OF PATIENT AT TIME OF ONSET IN RELATION TO HISTOLOGIC TYPE OF NEOPLASM

Age at time of onset	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
All ages (total)	390	286	83	39	26	18	21
0–9 yrs.	10	10	—	—	—	—	—
10–19	54	44	10	8	2	—	—
20–29	77	64	13	5	7	1	—
30–39	79	64	15	9	4	2	—
40–49	54	42	9	4	2	3	3
50–59	67	36	27	8	9	10	4
60–69	36	22	7	4	2	1	7
70–79	12	4	2	1	—	1	6
80 yrs. and over	1	—	—	—	—	—	1

TABLE 3. THYROID CARCINOMA: AGE OF PATIENT AT TIME OF INITIAL OPERATION IN RELATION TO HISTOLOGIC TYPE OF NEOPLASM

Age at time of initial operation	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
All ages (total)	390	286	83	39	26	18	21
0–9 yrs.	7	7	—	—	—	—	—
10–19	28	24	4	4	—	—	—
20–29	68	60	8	4	4	—	—
30–39	80	65	15	10	2	3	—
40–49	65	52	10	6	3	1	3
50–59	67	37	26	7	11	8	4
60–69	54	32	15	5	5	5	7
70–79	19	9	4	2	1	1	6
80 yrs. and over	2	—	1	1	—	—	1

TABLE 4. THYROID CARCINOMA: HISTOLOGIC TYPE AND EXTENT AT TIME OF INITIAL OPERATION

Histologic type	No. of patients	Extent at initial operation						
		One lobe	Both lobes	Isthmus only	Pyramidal lobe only	Thyroglossal duct area	Unknown (lymph node biopsy only)	Unknown (operation elsewhere)
All types (total)	390	294	74	11	1	1	3	6
Papillary	286	213	56	10	1	1	3	2
Follicular, total	83	67	11	1	—	—	—	4
Localized	39	38	1	—	—	—	—	—
Invasive	26	17	5	1	—	—	—	3
Medullary	18	12	5	—	—	—	—	1
Anaplastic	21	14	7	—	—	—	—	—

carcinoma appeared with greatest frequency among patients in the 20–39 year age group, follicular carcinoma among patients in the 50–59 year group, and anaplastic carcinoma among patients in the 60–69 year group. The majority of patients with the localized type of follicular carcinoma were less than 50 years of age, whereas the majority of those with the medullary type were 50 years of age or older.

Histologic type of carcinoma and extent of involvement of thyroid gland at initial operation

Both lobes of the thyroid gland were involved by thyroid carcinoma in 19 per cent of all patients at the time of the "initial operation" (Table 4). There were no significant differences between histologic groups as far as frequency of bilateral involvement was concerned ($\chi^2 = 2.32$ with 3 df.). (Localized follicular carcinomas were excluded from the calculation.) One localized follicular carcinoma that arose in the isthmus extended into both lateral lobes.

Histologic type and stage of carcinoma at initial operation

At the time of the "initial operation," the disease was considered to be confined to the thyroid gland in more than 50 per cent of the 390 patients and in those of each histologic group with the exception of medullary follicular and anaplastic carcinomas (Table 5). In 72 per cent of patients with medullary follicular carcinoma (13 of 18), the disease had already spread to regional lymph nodes. In 52 per cent of patients with anaplastic carcinoma (11 of 21), either regional lymph node (5 patients) or distant (6 patients) metastases had already occurred at the time of the "initial operation."

TABLE 5. THYROID CARCINOMA: HISTOLOGIC TYPE AND STAGE AT TIME OF INITIAL OPERATION

Histologic type	No. of patients	Stage at initial operation			
		Localized	Regional	Distant	Unknown
All types (total)	390	237	115	23	15
Papillary	286	174	93	10	9
Follicular, total	83	53	17	7	6
Localized	39	36	1	2	—
Invasive	26	14	3	4	5
Medullary	18	3	13	1	1
Anaplastic	21	10	5	6	—

Histologic type of carcinoma and the status of regional lymph node and distant metastases

Table 6 shows that regional lymph node metastases occurred in 42 per cent (162 of 390) of all patients, and that lymph node involvement occurred within two months of the "initial operation" in 33 per cent (127 of 390) of all patients. The frequency of "early" and "late" regional lymph node metastases was highest among patients with medullary carcinoma (94 per cent). In anaplastic carcinoma the frequency of "early" and "late" regional lymph node metastases was 52 per cent, and in papillary carcinoma it was 44 per cent.

Distant metastases were noted in 16 per cent (61 of 390) of all patients; in 6 per cent (23 of 390), distant metastases appeared within two months after the "initial operation" (Table 7). The frequency of "early" and "late" distant metastases was highest (50 per cent) among patients with invasive follicular carcinoma; the frequency among patients with anaplastic carcinoma was 43 per cent; with medullary carcinoma 39 per cent; with localized follicular carcinoma 13 per cent; and with papillary carcinoma only 9 per cent.

TABLE 6. THYROID CARCINOMA: STATUS OF REGIONAL LYMPH NODE METASTASES IN RELATION TO HISTOLOGIC TYPE OF PRIMARY NEOPLASM

Status of regional lymph node metastases	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
Total number of primary neoplasms	390	286	83	39	26	18	21
Metastases reported, total	162	126	25	2	6	17	11
Early	100	78	14	1	3	10	8
Late	35	25	7	1	3	3	3
Both early and late	27	23	4	—	—	4	—
No metastases reported	228	160	58	37	20	1	10

TABLE 7. THYROID CARCINOMA: STATUS OF DISTANT METASTASES IN RELATION TO HISTOLOGIC TYPE OF PRIMARY NEOPLASM

Status of distant metastases	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized Invasive		Medullary	
Total number of primary neoplasms	390	286	83	39	26	18	21
Metastases reported, total	61	27	25	5	13	7	9
Early	16	8	2	1	1	—	6
Late	38	17	18	3	9	6	3
Both early and late	7	2	5	1	3	1	—
No metastases reported	329	259	58	34	13	11	12

No significant relationships were found between the occurrence of regional lymph node metastases and of distant metastases, as shown in Table 8 ($\chi^2=2.13$ with 1 df.). It was noted, however, that almost half of the patients with distant metastases had no regional lymph node metastases.

Type of initial thyroidectomy by year of operation

A considerable change, especially in the last decade, has occurred in the type of thyroidectomy performed at the University of California Hospitals (Table 9). Unilateral or bilateral subtotal lobectomies were performed on 56 per cent of the patients (101 of 180) from 1930 to 1949, but in the last decade, 67 per cent (131 of 196) were treated by either total thyroidectomy (46 per cent) or total lobectomy on the side of the primary neoplasm combined with a subtotal lobectomy on the opposite side (21 per cent).

Type of lymph node operation by year of operation

Radical excision of lymph nodes was not performed on any of the

TABLE 8. THYROID CARCINOMA: STATUS OF REGIONAL LYMPH NODE AND DISTANT METASTASES

Status of regional lymph node metastases	No of patients	Status of distant metastases				No distant metastases reported
		Reported				
		Total	Early	Late	Both	
Total number of primary neoplasms	390	61	16	38	7	329
Metastases reported, total	162	31	8	21	2	131
Early	100	13	7	6	—	87
Late	35	10	—	9	1	25
Both early and late	27	8	1	6	1	19
No metastases reported	228	30	8	17	5	198

TABLE 9. THYROID CARCINOMA: TYPE OF INITIAL THYROIDECTOMY BY YEAR OF OPERATION

Type of initial thyroidectomy	No. of patients	Year of initial operation							
		1920–24	1925–29	1930–34	1935–39	1940–44	1945–49	1950–54	1955–59
Total cases of carcinoma	390	4	10	12	36	45	87	102	94
Thyroid biopsy only	3	—	—	—	1	—	—	1	1
Subtotal lobectomy									
Unilateral	49	1	3	4	6	13	9	9	4
Bilateral	108	2	6	3	15	17	34	16	15
Pyramidal lobe only	1	—	—	—	—	—	1	—	—
Isthmus only	1	—	—	—	—	—	—	1	—
Total lobectomy									
Unilateral	43	—	—	2	5	9	11	7	9
Unilateral subtotal and unilateral total lobectomy	61	—	—	1	3	1	16	20	20
Subtotal or partial thyroidectomy, not otherwise specified	6	—	1	—	2	2	1	—	—
Total thyroidectomy	108	—	—	1	1	1	14	47	44
Unknown	6	1	—	1	2	1	1	—	—
None	4	—	—	—	1	1	—	1	1

patients prior to 1940 (Table 10). During the past fifteen years there has been a steady increase in the number of patients treated by radical neck dissection, and a slight decline in the number of patients treated by modified neck dissection. During the past fifteen years, excision of the regional lymph nodes has not been included in over half (53 per cent) of the "initial operations."

Type of thyroidectomy and the frequency of local recurrence

Local recurrence in the thyroid area was observed in 13 per cent of patients (40 of 315) in whom the "initial operation" was considered to have completely removed the primary neoplasm from the gland (Table 11). The rate of local recurrence was lowest in those patients who were subjected to more extensive operative procedures. Recurrence rates ranged from 29

TABLE 10. THYROID CARCINOMA: TYPE OF INITIAL LYMPH NODE OPERATION BY YEAR OF OPERATION

Type of initial lymph node operation	No. of patients	Year of initial operation							
		1920–24	1925–29	1930–34	1935–39	1940–44	1945–49	1950–54	1955–59
Total cases of carcinoma	390	4	10	12	36	45	87	102	94
Lymph node biopsy only	14	—	2	—	2	—	2	2	6
Local excision of enlarged cervical node:									
a. Without mediastinal dissection	65	—	1	4	4	2	13	30	11
b. With mediastinal dissection	2	—	—	—	—	—	—	1	1
Modified neck dissection:									
a. Unilateral	14	—	—	—	—	2	6	2	4
b. Bilateral	5	—	—	—	—	—	4	1	—
Radical neck dissection:									
a. Unilateral									
1) without mediastinal dissection	35	—	—	—	—	3	9	10	13
2) with mediastinal dissection	4	—	—	—	—	—	—	2	2
b. Bilateral									
1) without mediastinal dissection	3	—	—	—	—	—	—	1	2
2) with mediastinal dissection	4	—	—	—	—	—	—	2	2
Unilateral modified and unilateral radical neck dissection	2	—	—	—	—	—	1	1	—
Mediastinal dissection only	1	—	—	—	—	—	—	1	—
Unknown	13	1	1	1	3	4	3	—	—
None	228	3	6	7	27	34	49	49	53

TABLE 11. SURGICALLY TREATED THYROID CARCINOMA: TYPE OF THYROIDECTOMY IN RELATION TO LOCAL RECURRENCE

Type of thyroidectomy*	No. of patients	Local recurrence reported					No local recurrence reported
		Total	On same side	On opposite side	On both sides	Side unknown	
Total cases surgically treated	315	40	26	3	4	7	275
Subtotal lobectomy							
Unilateral	34	10	7	1	1	1	24
Bilateral	85	9	5	1	1	2	76
Pyramidal lobe only	1	—	—	—	—	—	1
Isthmus only	1	1	1	—	—	—	—
Total lobectomy							
Unilateral	38	7	4	1	—	2	31
Unilateral subtotal lobectomy and unilateral total lobectomy	54	5	4	—	1	—	49
Subtotal or partial thyroidectomy, not otherwise specified	6	6	4	—	1	1	—
Total thyroidectomy	96	2	1	—	—	1	94

* Patients who underwent either an unspecified type of thyroidectomy or no thyroidectomy and patients with tumors known to be incompletely removed were excluded from the tabulation.

per cent (10 in 34) for patients treated only by unilateral subtotal lobectomy, to 2 per cent (2 in 96) for patients treated by total thyroidectomy. The majority of the local recurrences (26 of 40) appeared on the same side as the primary neoplasm, but in at least 7 known instances the opposite side was involved in the recurrence.

Postoperative radiation in relation to histologic type of carcinoma

Of the 390 patients with thyroid carcinoma, 113 received postoperative irradiation to the thyroid and cervical areas and to distant sites of metastases by means of x-rays, radioiodine, or both (Table 12).

Of 286 patients with papillary carcinoma, 46 received such postoperative irradiation within two months after the "initial operation." Some of these patients survived for long periods. Evaluation of radiation therapy however was not possible in this group of patients.

Survival rates

Thyroid carcinoma by histologic type. Patients with papillary carcinoma had significantly higher survival rates than those with follicular carcinoma at ten and twenty years after the onset of carcinoma, but there were no significant differences between the two groups at thirty and forty years (Fig. 1). Patients with papillary or follicular carcinoma had significantly higher survival rates than those with anaplastic carcinoma at ten and twenty years after onset.

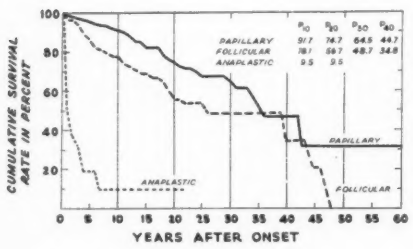

FIG. 1. Cumulative survival rates after onset for patients with thyroid carcinoma, according to histologic type.

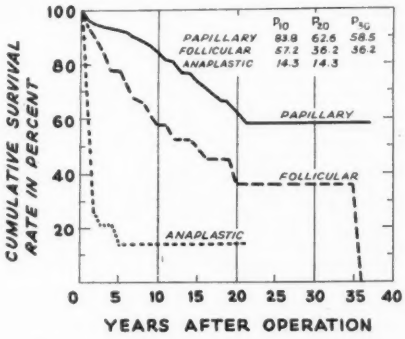

FIG. 2. Cumulative survival rates after operation in patients with thyroid carcinoma, according to histologic type.

When calculated from the time of the "initial operation," survival rates for patients with papillary or follicular carcinoma were significantly higher than for patients with anaplastic carcinoma at ten years (Fig. 2). Patients with papillary carcinoma had significantly higher survival rates than those with follicular carcinoma at ten and twenty years after operation, but there were no significant differences between the two groups at thirty years. No significant differences were noted between the survival rates of the follicular and anaplastic groups at twenty years.

Papillary carcinoma by sex of patient. No significant differences in sur-

TABLE 12. THYROID CARCINOMA: RADIATION THERAPY IN RELATION TO HISTOLOGIC TYPE OF NEOPLASM

Radiation therapy	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
Total cases of carcinoma	390	286	83	39	26	18	21
Radiation therapy received:							
Within 2 mos.*	74	46†	20	—	12	8	8
Two mos. to 1 yr.*	10	6	1	—	1	—	3
More than 1 yr.*	18	15	3	—	3	—	—
To distant metastases only	9	4	4	1	2	1	1
Date unknown	2	2	—	—	—	—	—
Preoperative radiation therapy only	6	5	—	—	—	—	1
None recorded	271	208	55	38	8	9	8

* After "initial operation."

† Includes 2 cases of lymph node biopsy only; 1 case of unilateral modified neck dissection only; and 3 cases of thyroid gland biopsy only.

vival rates were noted between the sexes when calculated from either the time of onset of the carcinoma or of the "initial operation."

Papillary carcinoma by age of patient. Patients under 40 years of age had significantly higher survival rates than those 40 years of age and over at ten, twenty and thirty years after onset of the carcinoma or after the "initial operation."

Papillary carcinoma by stage. At ten years after the "initial operation," the survival rates for patients with "localized" and "regional" disease were not significantly different, but both were significantly greater than that for patients with "distant" disease (Fig. 3). At twenty years, the "regional" group had a significantly higher survival rate than did the "localized" and "distant" groups. The latter two groups had survival rates which were not significantly different from each other at twenty years. The "regional" group had a higher survival rate than the "localized" group at thirty years.

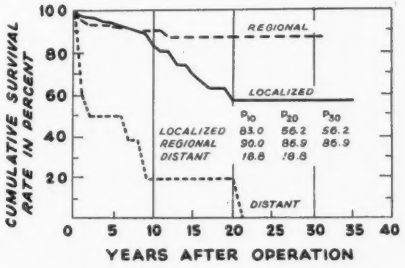

FIG. 3. Cumulative survival rates after operation for patients with papillary carcinoma, according to the stage of carcinoma.

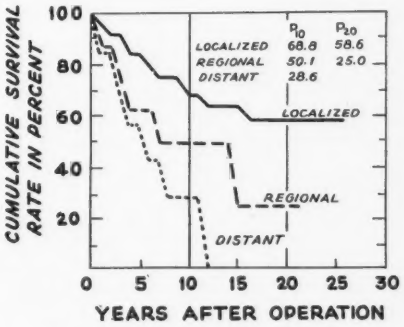

FIG. 4. Cumulative survival rates after operation for patients with follicular carcinoma, according to the stage of carcinoma.

Follicular carcinoma by sex of patient. The only significant difference in survival rates between the sexes was at forty years after the onset of carcinoma, when the survival rate of males was significantly higher than that of females.

Follicular carcinoma by age of patient. Comparisons of survival rates calculated from the time of onset of the carcinoma and from the time of the "initial operation" showed significantly higher rates for patients under 40 years of age, except at forty years after onset; at this time there was no significant difference between the two groups.

Follicular carcinoma by stage. The survival rate for the "localized" group

was significantly greater than that for the "distant" group at ten years after the "initial operation" (Fig. 4). No other significant differences were noted among the "localized," "regional" and "distant" groups.

Follicular carcinoma by subgroup. When calculated from the time of onset of the carcinoma, the survival rates for patients with "localized follicular carcinoma" were greater than those for patients with "invasive follicular carcinoma" at twenty, thirty and forty years (Fig. 5). No other significant differences were noted.

When calculated from the time of the "initial operation," the survival rates for patients with "localized follicular carcinoma" were greater than

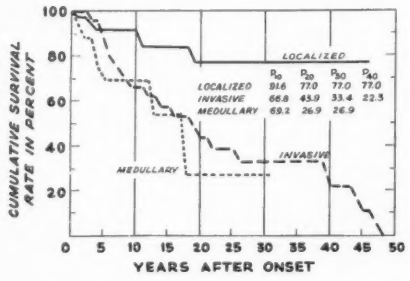

FIG. 5. Cumulative survival rates after onset for patients with follicular carcinoma, according to histologic subgroup.

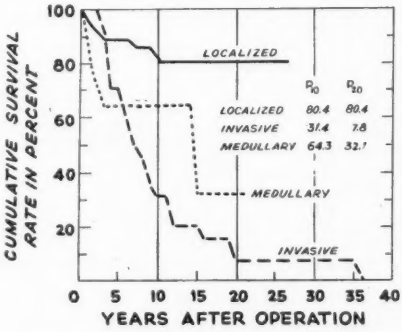

FIG. 6. Cumulative survival rates after operation for patients with follicular carcinoma, according to histologic subgroup.

those for patients with "invasive follicular carcinoma" at ten and twenty years (Fig. 6). At ten years, the "medullary carcinoma" group had a significantly higher survival rate than the "invasive follicular carcinoma" group. No other significant differences were noted.

Anaplastic carcinoma. Because of the small numbers of patients with anaplastic carcinoma, no additional survival rates were calculated.

Mortality data

Status of patients at time of the last report, by histologic type of carcinoma. For the total group of 390 patients, 25 per cent were reported dead from any cause. Table 13 shows that most patients with invasive follicular carcinoma (81 per cent, 21 of 26) and anaplastic carcinoma (81 per cent, 17 of 21) were dead at the time of the last report. Carcinoma of the thyroid gland was the cause of death in 19 per cent of the total group of 390 patients, and in 11 per cent of the papillary, 33 per cent of the follicular, and 76 per cent of the anaplastic groups.

TABLE 13. THYROID CARCINOMA: STATUS OF PATIENTS AT LAST REPORT IN RELATION TO HISTOLOGIC TYPE OF NEOPLASM

Status of patient at time of last report	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
Initial number of patients	390	286	83	39	26	18	21
Total alive	285	233	48	33	5	10	4
Without neoplasm*	169	145	24	15	3	6	—
With neoplasm	8	3	4	3	—	1	1
Status of neoplasm unknown	108	85	20	15	2	3	3
Total dead	98	47	34	6	21	7	17
Neoplasm the cause of death	75	32	27	2	18	7	16
Other cause of death (neoplasm not mentioned)	21	15	6	4	2	—	—
Other cause of death (neoplasm mentioned)	2	—	1	—	1	—	1
No follow-up	7	6	1	—	—	1	—

* Neoplasm refers to thyroid carcinoma.

Histologic type of carcinoma by sex of patient. Deaths among females outnumbered deaths among males by approximately 3:1 (Table 14). In the papillary carcinoma group, there were no significant differences in numbers of deaths among males as compared to females (17 per cent and 9 per cent respectively). ($\chi^2 = 2.75$ with 1 df.)

Age of patient by histologic type of carcinoma. Table 15 shows that for most histologic types, the median age of the patient at the time of death was in the 60–69 year range. Most of the deaths from medullary carcinoma, however, occurred in patients of the 50–59 year age group.

Duration of carcinoma in relation to histologic type. A tabulation of deaths due to thyroid carcinoma calculated according to the duration of the disease is shown in Table 16. The median duration of disease in pa-

TABLE 14. DEATHS DUE TO THYROID CARCINOMA IN RELATION TO HISTOLOGIC TYPE OF NEOPLASM AND SEX OF PATIENT

Histologic type	No. of deaths	Sex of patient	
		Male	Female
Thyroid carcinoma (total)	75	17	58
Papillary	32	12	20
Follicular, total	27	5	22
Localized	2	—	2
Invasive	18	4	14
Medullary	7	1	6
Anaplastic	16	—	16

TABLE 15. DEATHS DUE TO THYROID CARCINOMA IN RELATION TO AGE OF PATIENT AND HISTOLOGIC TYPE OF NEOPLASM

Age of patient at time of death	No. of patients	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
All ages (deaths)	75	32	27	2	18	7	16
Under 10 yrs.	1	1	—	—	—	—	—
10–19	—	—	—	—	—	—	—
20–29	2	2	—	—	—	—	—
30–39	4	1	3	—	2	1	—
40–49	6	2	2	1	—	1	2
50–59	16	5	8	—	4	4	3
60–69	27	10	11	1	9	1	6
70–79	16	9	3	—	3	—	4
80 yrs. and over	3	2	—	—	—	—	1

tients with medullary carcinoma was approximately three years. The longest duration of disease was noted in a patient with invasive follicular carcinoma who died forty-seven years after onset of the disease.

COMMENT

Although carcinoma of the thyroid gland occurs at all ages, the various types of thyroid cancer show distinct age distribution. As in other sorts of thyroid disease, females outnumber males by a considerable margin.

This study showed a high frequency both of bilateral involvement of the

TABLE 16. DEATHS DUE TO THYROID CARCINOMA IN RELATION TO DURATION OF NEOPLASM AND HISTOLOGIC TYPE

Duration of carcinoma at time of death	No. of cases	Histologic type of carcinoma					
		Papillary	Follicular				Anaplastic
			Total	Localized	Invasive	Medullary	
Deaths due to thyroid carcinoma	75	32	27	2	18	7	16
Duration of carcinoma:							
Less than 1 yr.	14	3	1	—	—	1	10
1 yr.	6	2	1	—	—	1	3
2 yrs.	3	1	1	—	1	—	1
3 yrs.	5	1	3	1	—	2	1
4 yrs.	3	1	2	—	1	1	—
5–9 yrs.	13	6	6	—	6	—	1
10–14 yrs.	13	9	4	1	2	1	—
15–19 yrs.	7	3	4	—	3	1	—
20–24 yrs.	5	4	1	—	1	—	—
25–29 yrs.	1	—	1	—	1	—	—
30–34 yrs.	1	1	—	—	—	—	—
35–39 yrs.	2	1	1	—	1	—	—
40–44 yrs.	1	—	1	—	1	—	—
45 yrs. and over	1	—	1	—	1	—	—

thyroid lobes at the "initial operation" and of the regional lymph nodes within a two-month period following the "initial operation." These data suggest that more radical surgical treatment of the thyroid neoplasm as well as of the regional lymph nodes is warranted. This therapeutic approach has been advocated in other reports (2, 6).

No significant relationships were demonstrated between the occurrence of metastases in regional lymph nodes and in distant sites, thus suggesting that the primary neoplasm rather than involved regional lymph nodes was the source of the distant metastases. This concept was reinforced by the finding that nearly half the patients with distant metastases did not have regional lymph node metastases. Nevertheless, metastatic disease in regional lymph nodes should be removed if the disease is to be eradicated.

It is noteworthy that during the past fifteen years the majority of patients have been treated by more extensive forms of thyroidectomy. The data from the present study show a significantly lower rate of local recurrence in the thyroid area in the patients treated by total thyroidectomy as compared with those treated by unilateral subtotal lobectomy. Although the patients treated by total thyroidectomy were observed mainly during the last fifteen years, local recurrence might be expected by the present time in many of them if it were to occur, since it was shown previously (2) that such recurrence in papillary carcinoma appeared on an average of 7.7 years after operation, in follicular carcinoma 4.5 years after operation, and in anaplastic carcinoma 1.7 years after operation. The high mortality from respiratory obstruction associated with local recurrence of carcinoma in the thyroid area may be significantly reduced by total thyroidectomy.

Analysis of survival data in the present study shows significantly higher survival rates for patients with papillary carcinoma as compared to those with follicular carcinoma, although at thirty years or more after onset or after the initial operation, these differences in survival rates disappear.

For at least thirty years after onset or after operation, there were no differences in the survival of males and females with either papillary or follicular carcinoma. As shown in a previous study (2), patients with either papillary or follicular carcinoma who were under the age of 40 years had higher survival rates than patients over 40 years of age, although these data were not adjusted for age according to a standard population. Other data, however, suggested that thyroid carcinoma is more aggressive in older persons (2).

When survival of patients with papillary carcinoma was analyzed according to the stage of the disease, it was found that at twenty and thirty years after the "initial operation" those with "regional" disease had higher survival rates than those with "localized" disease. This finding suggests

that regional nodal metastases may delay spread of disease, or that some papillary carcinomas may tend to spread through the lymphatics to the regional nodes, whereas others, perhaps more aggressive, may metastasize through the vascular system to distant sites instead.

That medullary carcinoma is an entity distinct from other types of invasive follicular carcinoma, statistically as well as histologically (3), is suggested by a significantly higher survival rate at ten years after the "initial operation" for patients with this form of thyroid carcinoma as compared with that for patients with invasive follicular carcinoma.

This study again indicates that thyroid carcinoma is a lethal process, although the course of most forms of the disease, with the exception of the anaplastic type, may be prolonged considerably. The delayed but aggressive characteristics of papillary carcinoma are evident, since 11 per cent of the patients with this type of thyroid cancer died eventually of the disease. Papillary carcinoma is more frequent in the younger age groups, yet death from papillary carcinoma or from any other form of thyroid carcinoma is uncommon in patients below the age of 50 years. Although it appeared in a previous study that significantly more males than females had died of papillary carcinoma (2), no such sex differences were found in the present study of a larger group of patients followed for longer periods of time.

The results of clinical, pathologic and statistical studies of the natural history of thyroid carcinoma therefore suggest that earlier and more radical surgical therapy aimed at cure rather than palliation should eventually lead to lowered mortality from this disease. Prolonged follow-up studies will be necessary to determine the validity of this thesis.

Acknowledgment

Grateful acknowledgment is made to Dr. Calvin Zippin and his staff of the General Tumor Registry for statistical assistance.

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Letter to the Editor

STEROIDOGENESIS IN THE HUMAN FETAL TESTIS: THE CONVERSION OF PREGNENOLONE-7α-H^3 TO DEHYDROEPIANDROSTERONE, TESTOSTERONE AND 4-ANDROSTENE-3,17-DIONE*

TO THE EDITOR:

A study of the pathways of steroid synthesis in the human fetal testis is interesting from the standpoint of understanding the mechanisms of the development of the male habitus of the fetus *in utero* and how the male embryo overcomes the influence of the mother's hormones during gestation. Insight into androgen steroidogenesis in the fetal testis may also help to explain idiopathic endocrinopathies and dysgenias of the urogenital system as observed postnatally.

Previous work has shown the capacity of human fetal testicular tissue to metabolize progesterone-4-C^{14}. The 17α-hydroxylating, 20α- and 20β-reducing, side-chain splitting, 17-keto reducing, and 21-hydroxylating enzymatic systems have been proved to be active in such tissues (1).

The testes of a 20-cm. (21 weeks) and a 23-cm. (24 weeks) fetus were separately incubated in Krebs-Ringer-phosphate-glucose buffer pH 7.4 at 37.5° C. for two hours in an atmosphere of 95% O_2-5% CO_2 together with ATP, DPN, TPN, DPNH, TPNH and 4.0 μc. (1.8 μg.) of pregnenolone-7α-H^3. The reaction was stopped with acetone, and the mixture was filtered. After removal of the acetone, the water phase was repeatedly subjected to extraction with ethyl acetate. The residues of the ethyl acetate extract were chromatographed on paper in the solvent systems benzeneformamide (diluted with methanol 1:1 v/v, hexane-benzene (1:1)-formamide (diluted with methanol 1:1 v/v), and methylcyclohexane-butanediol (undiluted) for various lengths of time.

Radioactive areas on the dried chromatograms were detected with a

* This work was supported by Grant A-3270, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Bethesda, Maryland.

4pi Atomic Accessories papergram scanner. The radioactive areas were eluted with methanol, and aliquots were subjected to acetylation and/or chromic acid oxidation and rechromatographed to purify and help identify the components.

Further chromatography of the radioactive materials in the systems methylcyclohexane-propylene glycol (diluted with methanol) and benzene-formamide (diluted with methanol) with minute quantities of nonradioactive dilutent carrier steroids, followed by scanning of the radioactive areas and spot tests for the carrier steroids, helped to decide the structure of each radioactive compound.

Finally, nonradioactive carrier was added to each component and from three to six crystallizations were carried out to constant specific activity.

TABLE 1. CONVERSION OF PREGNENOLONE-7α-H_3 TO $C_{19}O_2$ STEROIDS

Compound	Final total d.p.m. recovered*		Per cent conversion of added activity		Per cent conversion of total recovered activity	
	20-cm. fetus	23-cm. fetus	20-cm. fetus	23-cm. fetus	20-cm. fetus	23-cm. fetus
4-Androstene-3,17-dione	113,800	37,500	1.29	0.42	1.94	0.73
Dehydroepiandrosterone	224,680	466,130	2.55	5.29	3.75	7.78
Testosterone	1,911,360	837,620	21.72	9.51	22.08	9.58

* Average specific activity (d.p.m./mg.) may be derived by dividing each number by 5.

Acceptable limits for radiochemical purity were that three consecutive crystallizations and mother liquors deviated less than ± 5 per cent from the mean.

All counting was performed with internal standards in toluene-POP-POPOP in a Packard Scintillation spectrometer to an accuracy of better than 2 per cent.

Table 1 shows the observed disintegrations per minute and the specific activity of the dehydroepiandrosterone, testosterone and 4-androstene-3,17-dione found in the 2 testes incubations. From the data on percentage conversion, it is seen that both dehydroepiandrosterone and testosterone represent major end-products of steroidogenesis in these testes of second-trimester fetuses. It is also evident that the enzymatic system of side-chain splitting seemed to be active in the younger fetus (20-cm.), and that twice as much dehydroepiandrosterone was formed in the older one (23-cm.). However, no conclusions can be drawn from this work concerning the reality of these differences between the older and younger fetuses. Dehydroepiandrosterone is also a normal metabolite in fetal adrenal glands (2),

and recently has been found in adult bovine testes (3) as a metabolite of 5-pregnenolone.

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The Endocrine Society

The 1962 Annual Meeting

The Forty-Fourth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 21–22–23, 1962.*

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 22, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

The Chairman of Local Arrangements is Dr. Allan T. Kenyon.

All members are urged to make their hotel reservations immediately. The Palmer House will hold 300 bedrooms for members until May 1, 1962, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining for the A.M.A. meeting, please advise the hotel. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1962.

Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $15.00 for any part of the scientific sessions. Members will be charged a registration fee of $5.00.

Graduate students, residents and interns, and members of the medical departments of the armed forces will be admitted upon presentation of proper credentials CERTIFIED BY DEPARTMENT HEAD OR SUPERINTENDENT OF HOSPITAL and payment of $5.00 registration fee.

Final program, membership card and advance registration forms will be sent on May 15, 1962, to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send an original and four copies of the title and abstract to the Vice-President, Dr. Leslie L. Bennett, Dept. of Physiology, University of California Medical Center, San Francisco 22, California, *not later than February 15, 1962.* It is imperative that the abstracts be informa-

tive and complete with results and conclusions—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately two hundred abstracts submitted each year and compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee.

THE COUNCIL REQUESTS THAT AUTHORS ADHERE STRICTLY TO THE FOLLOWING RULES WHEN SUBMITTING ABSTRACTS, OTHERWISE THEY CANNOT BE CONSIDERED:

1. IT IS ASSUMED THAT ABSTRACTS SUBMITTED FOR THIS PROGRAM HAVE NOT AND WILL NOT BE SUBMITTED ELSEWHERE.
2. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas, graphs or extensive tables cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. Authors who wish receipt of their abstracts acknowledged should enclose self-addressed post card with the title of the abstract noted thereon.
5. Authors are also to enclose 3×5″ filing cards, one for each author, giving his name and initials in the upper right hand corner, and the complete title of the abstract with all the authors listed in the center of the card. There should be a second card enclosed listing the title and all authors and the mailing address of the senior author. This is necessary for indexing. Senior authors will be notified of the acceptance of their papers for the final program.
6. The body of the abstract, typed double space, should follow the heading. The original copy should be on bond paper. The original and four copies should be forwarded to Dr. Bennett.
7. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

The 1962 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $7,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Awards

The Council of The Endocrine Society has approved a change in status of the Upjohn Scholar to that of The Upjohn Awards.

There will be two awards in the amount of $1,250 each, one for the best article in format and content printed in Endocrinology and one in The Journal of Clinical Endocrinology and Metabolism during the preceding year.

Awardees will be chosen by the Awards Committee at its regular meeting in November of each year.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Obituary

FRANCIS MARION POTTENGER

To write an obituary for Frank Pottenger, a former president of our Society, is not a task nor an obligation, but an opportunity to be cherished, for I knew him long and well. He was a remarkable man with many fine attributes, but to my mind the most outstanding was his inexhaustible capacity for friendships. Never a medico-politician, he nevertheless had acquaintance with a truly fabulous number of physicians both in our country and abroad.

He travelled extensively throughout the length and breadth of our country and four times to Europe to study. He was prominently identified and interested in a host of medical organizations, many of which have, or will, pay him tribute. Though internationally recognized mainly as one of the great leaders in the understanding and treatment of tuberculosis, he was never the narrow specialist but diversely fascinated by many fields of internal medicine.

It was this gregarious friendliness with countless colleagues which proved so helpful to the establishment and pioneering of the first endocrine society in the world, "The Association for the Study of Internal Secretions," as it was named rather pompously and clumsily for many years. Having lived through the early precarious years of this organization, it may be stated frankly and truthfully that although "star names" such as Harvey Cushing, Lewellys Barker, Walter Cannon, Joseph Aub, E. C. Kendall, J. B. Collip and other early presidents lent prestige and attracted favorable attention to our society, it remains doubtful whether it would have survived had it not been for the faithful steadfast wisdom of Pottenger in matters of organization, finance and memberships.

Frank was our devoted secretary for fifteen years, and then President in both 1935 and 1936. It took courage to steer our ship through troubled waters, when clinical endocrinology was a dubious fumbling branch of medicine and its clinical exponents somewhat suspect.

Dr. Pottenger was born September 27, 1869, and lived to the wonderful age of 91, always indefatigable and alert. He provided to intimate audiences like the Pacific Interurban Clinical Club, where he was greatly beloved, increasingly stimulating dissertations in his later eighties! He was sober but enthusiastic, cheerful but steady, and indomitable.

The overwhelming majority of present members of The Endocrine Society never knew this man. This tribute is recorded therefore in part so that they may realize how much they are indebted to him for helping form and guide this now flourishing organization during its earlier tribulations.

He lived long and well.

H. Lisser, M.D.—Tenth President

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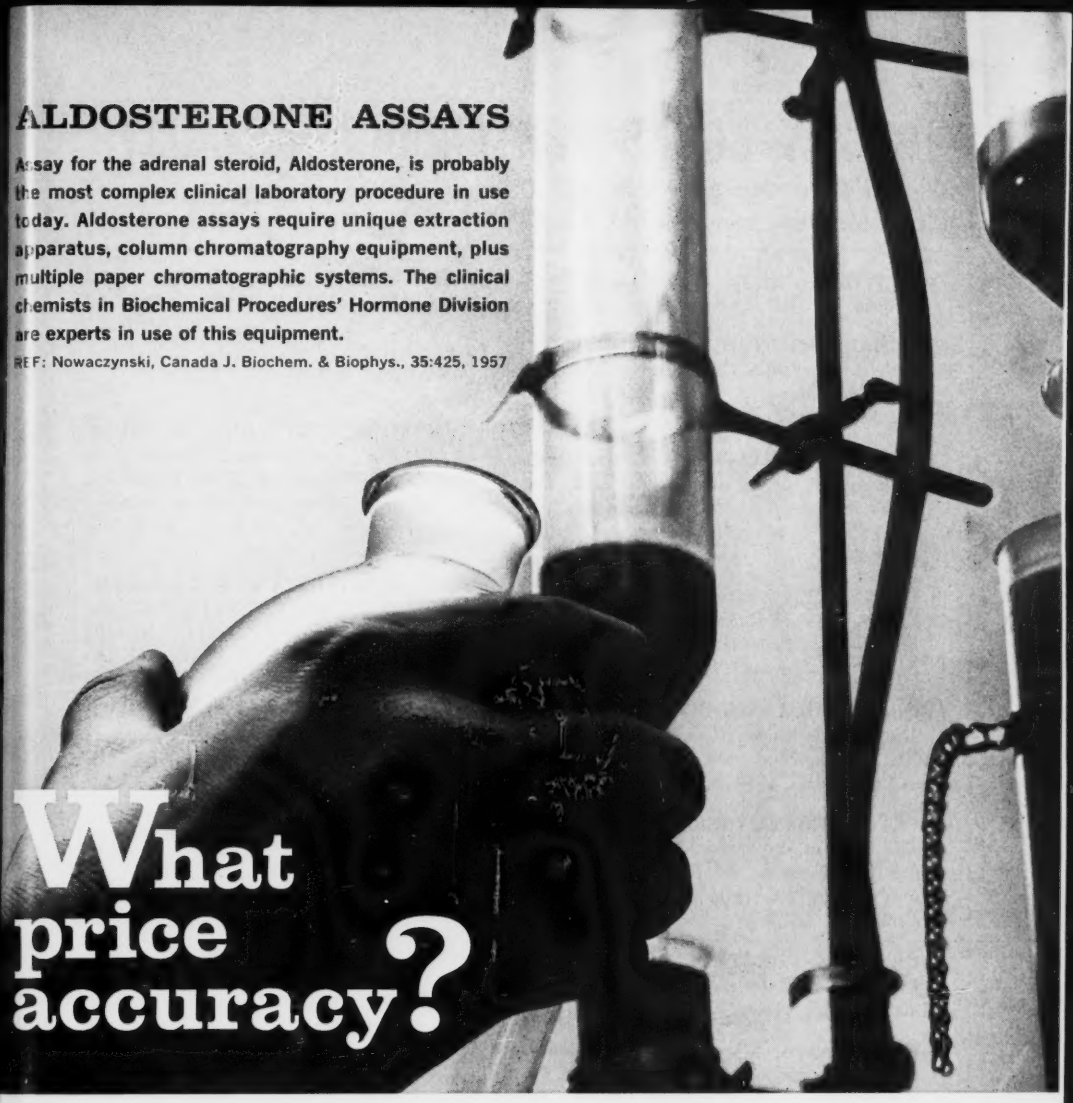

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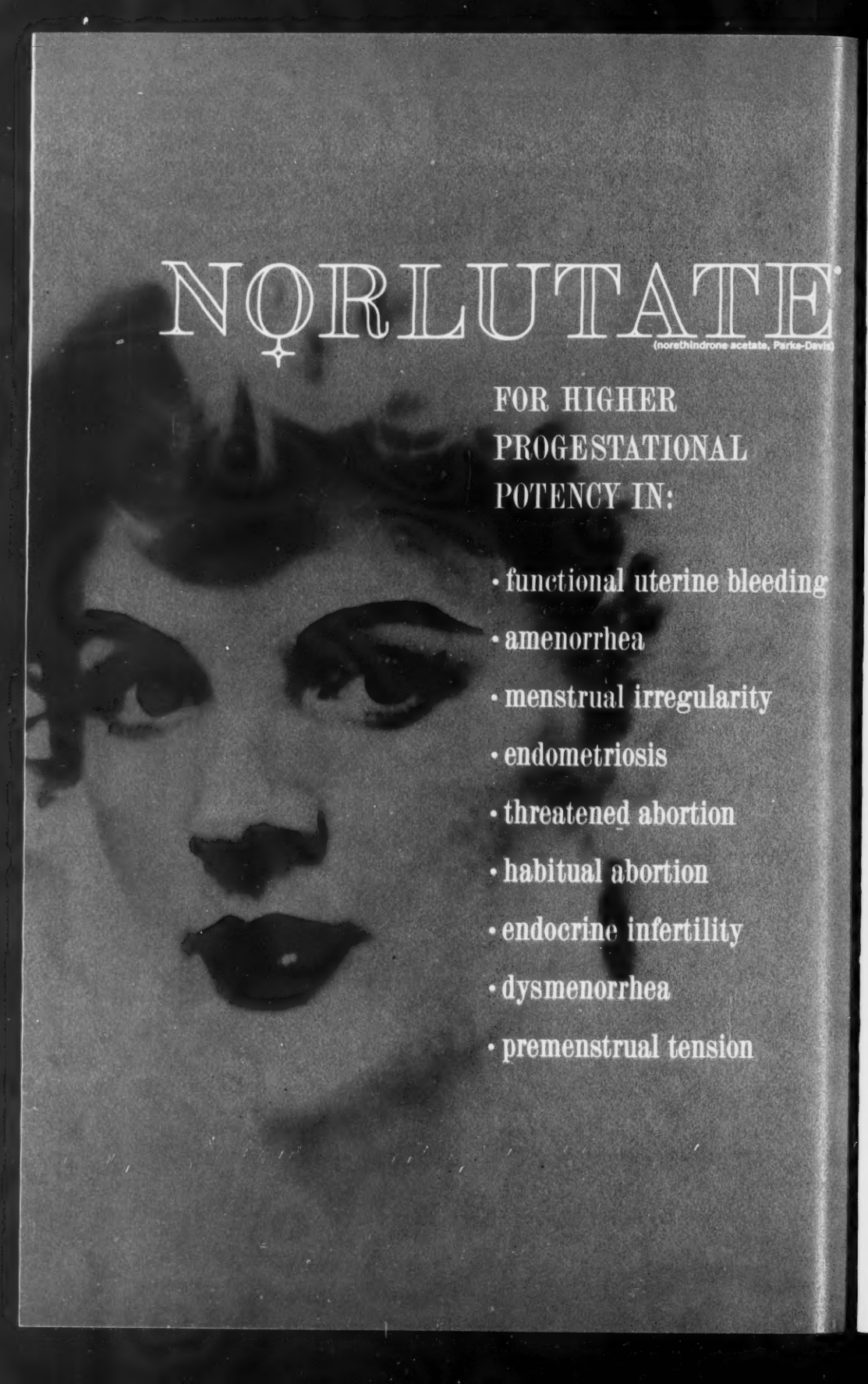
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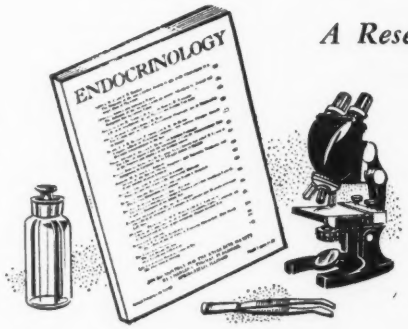

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